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# The University of Minnesota

Dept of
Pathology, bacteri
& Public
Health

CONTRIBUTIONS

FROM THE

# DEPARTMENTS OF PATHOLOGY AND BACTERIOLOGY

VOLUME II

1916-1919

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MINNEAPOLIS
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# CARCINOMA OF ESOPHAGUS WITH PERFORATION OF AORTA *

MOSES BARRON, B.S., M.D.
ST. PAUL

While carcinoma of the esophagus is by no means of infrequent occurrence, the case here described presents a particularly interesting complication. Although statistics vary, it is estimated that from about 5 (von Häcker) to 9 per cent. (McRae) of all cancers are esophageal. Cancer is by far the most important tumor of the esophagus.

## REPORT OF CASE

P. V. H., man, aged 58, admitted to the City and County Hospital, St. Paul, June 13, 1915, complaining of inability to swallow any solid food, stated that about two months before his admission a portion of meat which he had attempted to swallow seemed to lodge in his gullet and would not pass down into the stomach. He attempted to wash it down with a glass of water, but this resulted in both water and meat regurgitating through the nose and mouth. There was no pain present at this time. After this incident he was unable to swallow solid foods of any kind. The ingestion of acids, alkalies or other corrosive poisons was denied. Approximately eight months before this incident a fish bone had stuck in his throat, but after its dislodgment he had experienced no further trouble.

Shortly before his admission, a slight, dull pain appeared under the lower portion of the sternum; this would come on shortly after rising in the morning and disappear during the day. Although his appetite had continued good, his weight had steadily decreased from its normal level of 230 pounds to 190 pounds at the time of admission to the hospital. There was no history of tuberculosis or cancer in the family.

During his stay in the hospital, several attempts to introduce the stomach tube failed completely. Examination of the urine presented no abnormal features. The Wassermann reac-

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* Read before the Minnesota Pathological Society, Oct. 19, 1915.

* From the Department of Pathology, Bacteriology and Public Health, University of Minnesota Medical School, Minneapolis, and Pathological Laboratory, City and County Hospital, St. Paul.

tion was negative. Stools, July 31, gave a strong reaction for occult blood. Report on fluoroscopic examination of the stomach on June 18 reads as follows:

> The bismuth meets an obstruction at the lower part of the esophagus which bulges out the esophageal wall into a pouch from which bismuth can be seen flowing into the stomach like the sand in an hourglass.

A second fluoroscopic examination, July 22, gave a similar result. In order to eliminate the possibility of functional spasm, which had been suggested as an explanation of his symptoms, a hypodermic injection of 1/100 grain of atropin was given during the fluoroscopic examination without in any degree changing the result.

Temperature and pulse remained about normal. A few days before death the patient complained of pain in the abdomen. Seven hours before his demise he had a slight hemorrhage from his mouth. He became cyanosed, and his pulse perceptibly weakened. Three hours later there occurred a second hemorrhage with marked diminution of the patient's strength. The patient died fifteen minutes after a third hemorrhage.

I performed the necropsy fifteen hours after death. The essential features of the postmortem findings are as follows:

The body is very well developed and fairly well nourished. Opening the peritoneal cavity revealed the stomach and duodenum enormously distended, and discolored by their contents a dark purplish hue. The stomach, on being opened, is found to contain about 4 liters of fluid and clotted blood. The duodenum and intestinal tract also contain large quantities of fluid and clotted blood which, in the lower portion of the bowel, are tarry in color and consistence. The lower half of the esophagus presents a tumor mass which on its posterior surface infiltrates the aorta, near the junction of the transverse and descending portions of the arch. Several lymph nodes at the bifurcation of the trachea are enlarged to about the size of hazelnuts, firm in consistence, and of grayish color on cross section. A large nodular tumorous mass (Fig. 1, *e*), about 5 cm. in diameter, lies in the region of the lesser curvature of the stomach. On cross section this mass has a softened necrotic center, semifluid in consistence and yellowish in color. The posterior surface of the left lobe of the liver lies in contact with this mass, and is adherent to it in two places. Over these areas the capsule of the liver is apparently infiltrated, and on section this line of infiltration averages about 2 mm. in thickness.

The esophagus, trachea and aorta are removed *en masse*. When the esophagus is opened, the lumen of the lower half (Fig. 1) is found irregular, nodular, ulcerated, very ragged and necrotic, and reddish-brown. There is apparently no stenosis present. The involvement is annular, beginning just at the level of the bifurcation of the trachea and extending downward for a distance of 13 cm. The upper margin (*a*) is indurated, raised, and somewhat undermined. The lower margin (*b*) is irregular, and has a thickened overhanging edge, which on the average is about 1 cm. above the cardiac orifice, excepting posteriorly, where the uninvolved area

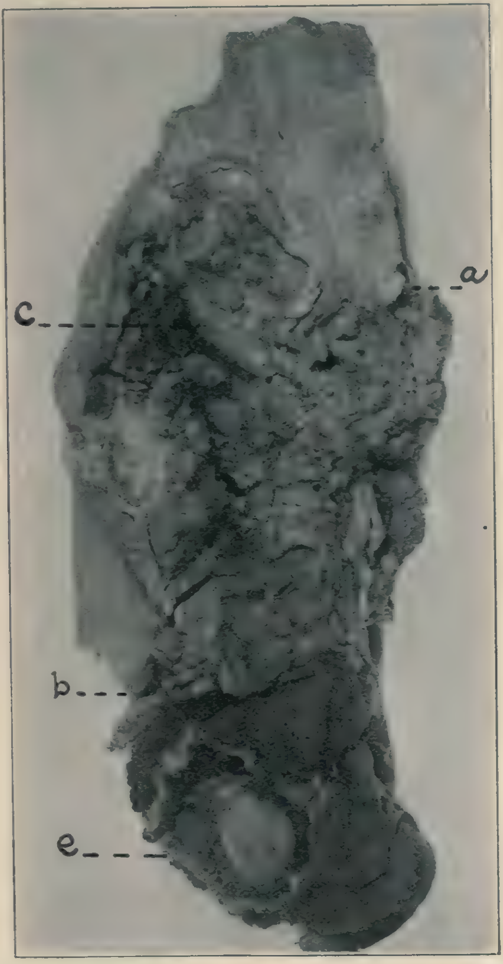

Fig. 1.—Lower half of esophagus opened, showing the ulcerated tumor mass. The perforation is at the mass of blood clot (*c*). The darker portion at the bottom is the cardia of the stomach.

tapers to a point about 5 cm. above the orifice. At the upper end of the tumor posteriorly, a small ragged mass of blood clot (*c*) infiltrates the necrotic wall. Opening of the aorta

(Fig. 2) reveals two small necrotic areas (*d*), each about 5 mm. in diameter and about 1 cm. apart, situated 3 cm. distal to the scarring of the obliterated ductus arteriosus. These necrotic areas, on being carefully probed, are found to be perforations communicating with the posterior wall of the esophagus in the region of the aforementioned adherent blood clot.

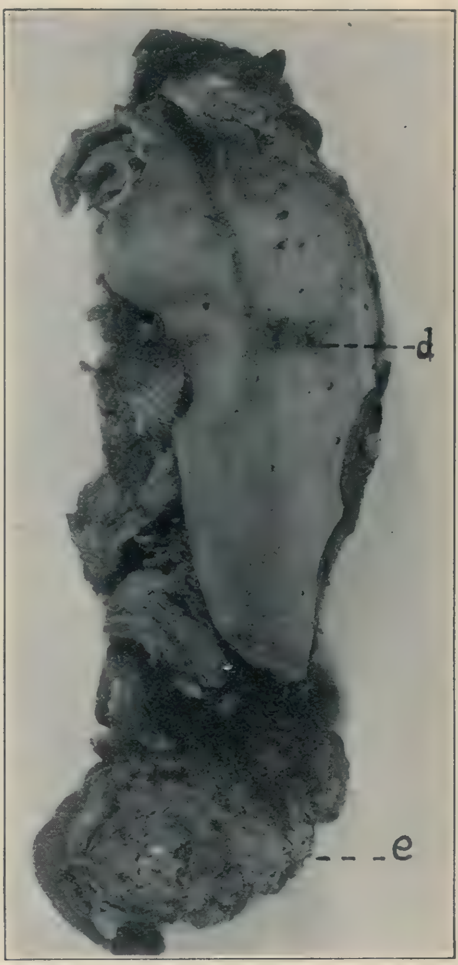

Fig. 2.—Same mass as in Figure 1, but seen from behind, showing the aorta. At *d* are the two perforations of the aorta. At *e* is a mass of enlarged infiltrated lymph nodes, seen also in Figure 1 *e*.

Microscopically, the tumor is composed of a very loose connective tissue stroma through which are scattered epithelial cells arranged in small groups, cords, and interlacing bundles, showing extensive cornification. These collections of cornified epithelial cells, though very numerous, are individually very small, and the "epithelial pearls" thus produced differ from the "pearls" in skin cancer not only in their smaller size but also in the less complete cornification of the centers. There is almost complete destruction of the muscular layers of the esophagus throughout the entire extent of the tumor.

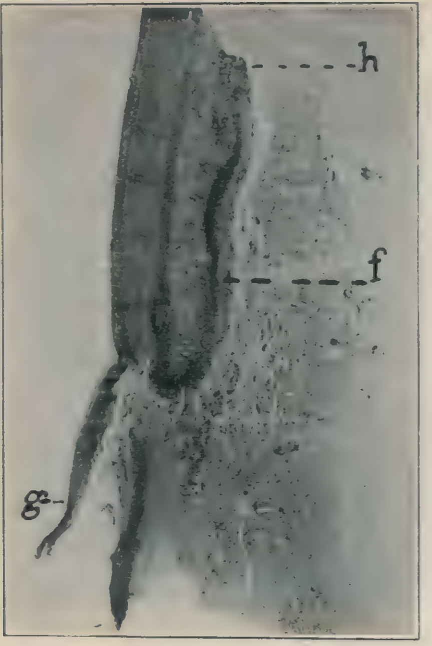

Fig. 3.—Low power photomicrograph of section taken at point *d*, Figure 2. The perforation is at the lower edge. At *g* only the intima is present. It is necrotic and infiltrated with large numbers of bacilli. The media and adventitia are entirely destroyed. At *f* is a layer of exudate on the adventitia composed of fibrin, cellular débris, leukocytes and bacteria.

Areas of necrosis are numerous, and around these there is an extensive infiltration of both polymorphonuclear leukocytes and lymphocytes. Section of the aorta at one of the points of perforation (Fig. 3) shows marked necrosis accompanied by leukocytic infiltration of all three coats. An extensive exudate of fibrin, leukocytes and bacteria is present on the adventitia (*f*). The intima shows various stages of degeneration and necrosis, accompanied by infiltration of numerous leukocytes (*g*). In these necrotic areas there are present an exceedingly large number of bacilli, which resemble morphologically the Oppler-Boas type.

### COMMENT

Just as in all cancers which are exposed to infection, the presence of secondary processes in this case seems to have contributed largely toward bringing about the final fatal outcome. It is rather noteworthy that in the microscopic sections relatively few tumor cells can be found invading the adventitia of the aorta (*h*), while the media and intima are apparently entirely free from invasion. Since the amount of infection is relatively great, and the amount of cancerous infiltration small, it is logical to conclude that the destructive inflammatory processes which accompanied the ulcerating tumor greatly contributed in bringing about the final perforation of the aorta. To these factors, of course, must also be added the results of the blood pressure within the aorta and the destruc-

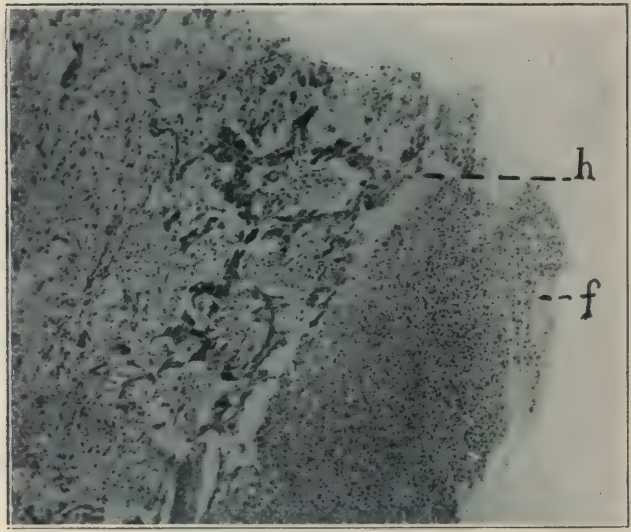

Fig. 4.—High power photomicrograph of section shown in Figure 3, showing the slight carcinomatous infiltration of adventitia at *h*. The inflammatory exudate is seen at *f*.

tive effects of the boluses of food passing down the gullet.

It certainly appears true, however, that, in this location, serious sequelae may result from cancerous infiltration, which is relatively much less extensive than that necessary to bring about equally grave results, for example, in the stomach or intestines. This may be explained by the fact that, in the latter organs, the infective processes are accompanied by a greater protective reaction on the part of the connective tissues.

## ETIOLOGY

Carcinomas of the esophagus develop principally at the physiologic constrictions of this organ,[1] that is, at the cricoid cartilage, at the bifurcation of the trachea (most frequently), and at the diaphragm. Foreign bodies, and chronic irritations from alcohol and tobacco[2] associated with some cellular predisposition[1] have all been suggested as etiologic factors. Men are much more frequently affected than women, in Kaufmann's series the ratio being seven men to one woman. Aschoff states that carcinoma of the esophagus is about five times more frequent in the male than in the female.

As with carcinomas in other locations, carcinoma of the esophagus generally occurs in people past middle age, most frequently during the sixth decade. It is rare before the age of 40, although Kaufmann notes Heimann's case of esophageal cancer in a girl 19 years old. He also cites a case of his own in a girl 21 years old in whom a small ulcerating squamous cell carcinoma was found in the upper part of the esophagus with large metastases into the lymph nodes of the neck. J. B. Cook[3] reports a case of epithelioma of the esophagus in a woman, aged 26, who had been losing flesh for a period of about three months, and had great difficulty in swallowing even liquids. No bougies could be introduced. At necropsy, carcinoma of the esophagus was found beginning in the region of the cricoid cartilage and extending downward for a distance of about 4 inches. It appears that cancer in this location, that is, in the region of the cricoid, affects women more often than men, and that it occurs much earlier in life.

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[1] Aschoff: Pathologische Anatomie, ii, 726.

[2] Kaufmann: Specielle pathologische Anatomie, Ed. 6, i, 410.

[3] Cook, J. B.: Brit. Med. Jour., 1909, i, 1416.

Steward and Mollison[4] report twenty-two cases, of which nineteen were in men and three were in women. The ages of the women were 22, 35 and 35, respectively, while of the men the ages ranged between 37 and 82, with an average of 58.5 years. Ulceration of the growth into the trachea with resulting pneumonia was the most frequent cause of death in this series. Nearly all patients complained of pain in swallowing, while the most constant symptom was the inability to swallow solids. Roentgen examination gave practically no assistance in the diagnosis, and, indeed, in several instances was directly misleading.

## PATHOLOGY

Aschoff[1] classifies the carcinomas of the esophagus in three main groups:

1. *Scirrhous.*—This type is nodular, infiltrating, and generally produces stenosis. Metastases may be absent, though sometimes these are extensive, even where the primary lesion is very small. Microscopically, there are narrow cords of a basal cell type of epithelial cells surrounded by dense bands of connective tissue.

2. *Medullary.*—This type shows a larger growth with extensive ulceration and necrosis. (The case under consideration belongs to this group.) Microscopically, there are broad cords of cornified epithelial cells. Numerous areas of softening and necrosis are usually present.

3. *Papillary.*—This type is composed of nodular papillary masses having broad bases. Central ulcerations may occur. Microscopically, the picture frequently presents a mixture of cylindric cells, squamous cells, and adenocarcinomatous formations.

Charles Hope[5] reports a case of columnar cell carcinoma of the esophagus in a man, aged 25. The patient had dysphagia and marked emaciation. At the postmortem a growth was found 5 inches long which extended down into the cardiac end of the stomach for a distance of one-quarter inch. There was a fistula present leading into the pericardium, where a large quantity of pus was found. There was also an extensive double empyema present. Secondary cancerous deposits were found in the liver. In this case there was a question as to whether the tumor, being a columnar cell type, did not originate primarily in the stomach and spread upward, instead of originating in the esophagus and spreading to the stomach. The doubt as to the primary location of the tumor is well founded, first, because the columnar cell type of tumor is rare in the esophagus (Hewlett cites six cases) but common in the stomach; and, second, because carcinoma of the esophagus rarely spreads downward into the stomach. It generally stops just at or above the orifice.[6] Some authors assert that many of the cancers in the lower part of the esophagus which involve the cardia are most probably primary in the stomach.

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[4] Stewart and Mollison: Guy's Hosp. Rep., London, 1912, lxvi, Series iii, 51.

[5] Hope, Charles: Proc. Roy. Soc. Med., 1911-1912. Laryngological Section, 5, Part 2, p. 84.

## SYMPTOMS

The most constant symptom is the inability to swallow solid food. This difficulty is not due entirely to the stenosis, which may be present, but is undoubtedly due partly to the destruction of the muscle fibers by the infiltrating growth. This is certainly well illustrated in the present case. It is important to note in this connection that the patients suffering from simple or functional spasm emphasize mostly their difficulty to swallow liquid foods, while those suffering from organic or true obstruction complain principally of the great distress brought on by attempting to swallow solid foods. That is, in simple spasm they regurgitate mostly liquids, while in organic obstruction the liquids may be swallowed with ease, but the solids may not pass down into the stomach at all.

Pain in swallowing is a very frequent complaint, but comes on probably only after the growth is already well developed. Lewisohn[7] asserts that cancer of the esophagus is a most insidious disease, and that early symptoms of anorexia, oppression in chest, burning pains, etc., may be present for several months before any difficulty in swallowing sets in. He states that esophagoscopy is of the greatest value in diagnosing these cases early.

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[6] Pepler, W. H.: Canadian Jour. Med. and Surg., 1909, xxvi, 345.

[7] Lewisohn: Med. Rec., New York, 1913, lxxxiv, 65.

## COMPLICATIONS

The regurgitation of food material mixed frequently with necrotic tumor masses, together with the progressive weakening of the patient, explains the frequency of death by aspiration pneumonia and gangrene of the lung. Perforation of the trachea or bronchi also results in aspiration pneumonia. There may also be infiltration with perforation in the pleural and pericardial cavities, resulting in purulent serositis, pneumothorax, etc. Perforation of the aorta (or of other blood vessels) results in sudden death through hemorrhage. Kaufmann[2] encountered only four such complications in his series of 126 cases of carcinoma of the esophagus. Levy Valensky[8] describes a case of esophageal cancer with perforation of the aorta. The patient spat blood, and a clinical diagnosis of central pneumonia was made. At necropsy an ulcerated carcinoma was found with two perforations into the aorta. A careful review of the literature shows the great rarity of such a complication. There may also be destruction of the bodies of the vertebrae with compressions of the spinal cord, giving rise to deformities.

Metastases are not very frequent excepting into the surrounding lymph nodes. It is somewhat peculiar that large tumorous growths may show few or no metastases, while small carcinomas may show extensive secondary deposits. The liver and the lungs are sometimes secondarily involved. I have examined at necropsy two other cases of carcinoma of the esophagus, since the present case was studied. One case showed only the faintest gross evidences of carcinomatous involvement of the esophagus. Indeed, only a microscopic study made the diagnosis definite. The metastases, however, were very prominent, showing large carcinomatous nodules in liver, spleen, kidneys, lungs and pleura. The other cases presented an annular carcinoma at the tracheal bifurcation. The stenosis was almost complete. The esophagus was infiltrated and thickened to about 1.5 cm. No metastases were present, even in any of the surrounding lymph nodes. The patient in this case died of starvation, being ultimately unable to swallow even liquids except in the minutest quantities. Borrman[9] cites a very interesting case of three apparently primary tumors of the esophagus with metastases into many organs, including fourteen pea-size tumor nodules scattered through the intestinal wall from jejunum to sigmoid. These nodules were situated principally opposite the mesenteric attachment, and the overlying mucosa was everywhere intact. Microscopically, all of the tumor masses were of the squamous cell type found in the esophagus. He concludes from his careful studies that these metastases must have occurred through the blood stream. He rules out the lymphatics as the route of dissemination because of the disposition of the tumors (opposite the mesenteric attachment) and because the mesenteric lymph nodes everywhere were uninvolved. He rules out implantations (a condition which he considers exceedingly rare) by the fact that the overlying epithelium was intact. Also, according to Bucher,[10] the masses of tumor tissue which break away from the primary growth are generally extensively necrotic, and it is hard to conceive that such tissues could become implanted on a healthy epithelium. Borrman was convinced that the middle tumor (at the tracheal bifurcation) was the true primary tumor, and that the upper one (at the cricoid) and the lower one (near the orifice and involving the cardia) were only metastases.

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[8] Valensky, Levy: Bull. et mém. Soc. anat. de Paris, 1910, lxxxv, 140.

## DIAGNOSIS AND TREATMENT

Roentgenoscopy does not prove very satisfactory in these cases, according to most authors. Libman[11] cites a case of carcinoma of the esophagus with perforation into the trachea which was diagnosed as tuberculosis. The fluoroscopic picture in this case helped to mislead in the diagnosis. The bronchoscope is of greater value, especially in early cases, but even then only in the hands of those skilled in the use of the instrument.

If diagnosed early, the treatment of the upper and lower tumors of the esophagus is surgical. Those in the region of the bifurcation are much less accessible. Torek,[12] however, describes a successful case of resec-

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9. Borrman, Robert: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1910, xlviii, 576.

10. Bucher, Robert: Beitr. z. path. Anat. u. z. allg. Path. (Ziegler's), 1893, xiv, 71.

11. Libman: Proc. New York Path. Soc., 1906-1907, N. S. 6-7, p. 17.

12. Torek: Surg., Gynec. and Obst., 1913, xvi, 614.

tion of the thoracic portion of the esophagus in a woman aged 67.

Many patients obtain temporary relief from the careful and systematic passage of bougies and esophageal dilators. This makes swallowing much less difficult. Gastrostomies are sometimes performed to help prolong life in cases in which the stenoses are very marked.

## CONCLUSION

I wish to emphasize several points relative to esophageal cancer:

1. Perforation of the aorta is relatively rare.

2. The bronchoscope is of great value for early diagnosis, but must be used with extreme caution in the advanced stages.

3. The almost general complaint of patients affected with esophageal cancer is their inability to swallow solids, in contradistinction to the regurgitation of liquids complained of in cases of simple spasm. In the case reported above, the onset of this symptom was sudden, occurring long before pain developed, and it persisted to the end.

4. It is important to bear in mind the dangers of esophageal manipulations with bougies, stomach tubes, dilators and esophagoscopes in cases of suspected carcinoma of the esophagus. Such examination in the later stage of the present case would most likely have resulted in traumatic rupture of the aorta with sudden fatal hemorrhage.

5. Fluoroscopy and the Roentgen ray are of undoubted value in the diagnosis of tumors in the lower part of the esophagus, but are of little value and frequently misleading in tumors of the upper part.

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# A HIGHLY DIFFERENTIATING POLYCHROMATIC TOLUIDIN-BLUE STAIN

By

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# A HIGHLY DIFFERENTIATING POLYCHROMATIC TOLUIDIN-BLUE STAIN*

By Moses Barron, M.D., Minneapolis, Minn.

Anyone working in a clinical-pathologic laboratory realizes the need for reliable stains that possess the virtues of precise differentiating powers, as well as simplicity and rapidity of technic. The almost countless number of stains that go to make up the armamentarium of the laboratory technician testify to the continuous efforts of many workers towards discovering serviceable stains.

For many years, I have been impressed with the polychromatic qualities of toluidin-blue. I became acquainted with the dye as an ingredient of Ponder's stain for diphtheria bacilli. I tried various combinations to increase its polychromatic properties, until one of our laboratory technicians, Richard Lundquist, suggested the idea of boiling the dye with an alkali. Heretofore, this process was used chiefly in obtaining various color compounds from methylene blue. For instance, Unna's polychrome methylene blue is prepared by boiling methylene blue with potassium carbonate. Similarly, the production of various azures is described by Tribondeau.[1] Goodpasture[2] describes a serviceable acid polychrome stain. But the polychromatic qualities obtainable from toluidin-blue seem to excel all the others in the sharpness of differentiation. Although its special usefulness lies in staining frozen sections for rapid diagnosis, it is of distinct value in a large number of routine laboratory procedures.

The preparation of the stain is as follows:

| | |
|---|---|
| Toluidin-blue | 1.0 |
| Potassium carbonate | 1.0 |
| Distilled water | 400.0 |

Boil solution in glass beaker until it is reduced to 300 c.c.
Cool; then add:

| | |
|---|---|
| Toluidin-blue | 2.0 |
| Sodium chloride | 3 0 |
| Glacial acetic acid | 12.0 |
| Alcohol | 15.0 |

Stir mixture until ingredients are completely dissolved. It is ready for use at once, and can be used over and over again. It does not precipitate and keeps indefinitely.

The following technic is employed in staining frozen sections for rapid diagnosis. A piece of tissue which is not more than 3 mm. in thickness is dropped into boiling 10 per cent formalin for about one minute. Fresh tissue without hardening may be used in many cases but the sections are more difficult to handle. The tissue is cut with the freezing microtome, and the sections are placed in a dish containing physiologic salt solution. The sections are quickly straightened out and carried over into a small dish of the stain where they are left for about fifteen seconds. They can be left for a much longer time without overstaining. The sections are then transferred to another dish of physiologic salt solution where they are rapidly washed for a few seconds to remove the excess of stain, and then are at once mounted in the same solution on a slide, and a coverslip applied. The section is now ready for examination. The entire process, from the time the section is cut to the completed preparation for microscopic study, need not take more than one minute in most instances.

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*From the Laboratory of Pathology of the Medical School of the University of Minnesota, Minneapolis, Minn.

I have found that by handling the sections in physiologic salt solution and by having approximately 1 per cent salt in the stain, the outlines of the cells and their nuclei are clearer and sharper in the resulting preparation. The acetic acid in the stain helps to accentuate the nuclei, while the alcohol aids in straightening out the section after its transference into the last salt solution.

Sections stained by this method give approximately the following microscopic pictures. The cytoplasm of epithelial cells stains a purplish blue of varying intensity depending upon the age of the cell. The younger the cell, the deeper the stain. The stratum germinativum of the skin, therefore, takes a deeper stain than the stratum corneum. The nuclei stain deep purple. Tumor cells, like those of carcinomata, stain deep purplish blue. Mitotic figures show up very distinctly, as the chromosomes stain a purplish black. Hyaline connective tissue stains pink and the intensity of the color is proportional to the degree of hyalinization present. The nuclei of the leucocytes take a very deep purple, while the cytoplasm stains only feebly bluish. The granules of the neutrophiles stain faintly bright green. The granules of the basophiles are dark purple, almost black. Plasma cells are easily identified. The cytoplasm is a light purplish blue, while the excentric nucleus appears reticular and takes a deep purple color. An achromatic zone surrounds the nucleus. Erythrocytes do not stain but appear greenish yellow. The endothelial cells lining blood vessels and lymph spaces stain light bluish purple.

The myelin sheaths of nerve fibers are bright purple, while the cytoplasm of the cells is bluish green. Cartilage stains a deep purple. Elastic fibers are brilliant green. Cells containing fat droplets present a dirty brown appearance. Because of this the zones in the lobules of a liver involved in chronic passive congestion with fatty metamorphosis stand out very prominently. In the adrenal, the cortex appears brown while the medulla is light blue.

Atherosclerosis of the aorta gives a beautiful picture. Atheromatous patches containing fat droplets are brown; elastic fibers are brilliant bluish green; and hyalinized connective tissues are pink.

Besides the staining of tissues, the examination of stools for ova and parasites is facilitated by the use of this stain. A drop of emulsified stool is mixed with a drop of the stain on a slide, and a coverslip applied. Practically all the elements in the normal stool take a purplish red color. If ova of any of the tenia be present, they stand out as regular yellowish or golden yellow bodies against a purple background. The identification of the ova thus becomes a simple matter because they appear so different from any other elements present.

But the stain is even more valuable for identifying amebæ in stools. Hitherto a correct diagnosis of ameba could be made only through the examination of a warm stool on a warming stage. With this stain, amebæ can readily be identified by taking a drop of the mucoid portion of the stool and mixing it with a small drop of the stain. The cytoplasm of the amebæ takes on a purplish blue and the outline of the cells becomes very sharp. The nucleus often shows very distinctly. Vacuoles and ingested particles become prominent. The amebæ can be recognized with the low power by their size, regularity in form, sharpness of cell outline, and the deeper bluish tinge of the cytoplasm than that of most of the other elements present. The absolute identification can then be made by means of the high power dry lens.

Diphtheria bacilli show up excellently with this stain. The smear is flooded with the stain and steamed gently for about ten to fifteen seconds. It is then washed and dried. The granules appear a deep brilliant purplish red, while the rest of the cell bodies stain a light purplish blue.

Because of the simplicity and the quite general usefulness of this stain I feel justified in reporting its formula.

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[1] Tribondeau, L., and Dubreuil, J.: Nouveaux colorants microscopie derives du bleu de methylene, Compt. rend. Acad. d. sc., 1917, clxiv, 551-53.

[2] Goodpasture, C. W.: An Acid Polychrome Methylene Blue Solution for Routine and Special Staining, Jour. Am. Med. Assn., 1917, lxix, No. 12, p. 998.

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# MENINGITIS IN THE NEWBORN AND IN EARLY INFANCY.

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Introduction. Although meningitis is a disease quite common in childhood, it is one that is exceedingly rare during the early months of infancy. In making a rather careful review of the literature, I have been able to find only 39 cases reported in infants under three months old. Of these 39 cases, 19, including the case to be reported in this paper, were in the newborn.

The etiology in these cases was interesting, especially in the newborn. Of the 19 cases in this series, 7 were caused by the same type of bacillus which was isolated in pure culture from the present case, the Bacillus coli; 6 cases were caused by the staphylococcus and streptococcus. The infection of most of these was through a spina bifida, a condition present in nearly every one of this group. Two were caused by the pneumococcus and one each by meningococcus, Bacillus mucosus capsulatus, Bacillus lactis aërogenes and Bacillus pyocyaneus.

The relatively frequent occurrence of colimeningitis among the newborn is emphasized by Allard,[1] who states that this is the commonest form encountered. He believes that infants and children are specially predisposed to meningeal diseases. Moll[2] claims that colimeningitis in nurslings is generally consequent to colicystitis. This statement is not substantiated by a careful study of the literature. Indeed, it is definitely stated in several cases that examination of the urine showed nothing abnormal and that the specimens were sterile.

Holt[3] reviews a series of 300 of his own cases of meningitis in infants up to three years old. The Bacillus tuberculosis was responsible for 70 per cent. of this series, but only 1 per cent. was in infants under three months old. He shows by statistics from the city of New York and from his own practice that if epidemic meningitis be excluded, from 55 per cent. to 70 per cent. of all cases of meningitis in infants and young children are tuberculous in origin. Practically no cases of colimeningitis are reported in children over six months old. Smith[4] states that 75 per cent. of all cases of tuberculous meningitis in children occur before the fifth year. The largest number occur during the second year. He also mentions the fact that, exclusive of epidemic meningitis, 70 per cent. of all meningeal infections are tuberculous. These statistics, together with the tables to follow, help to emphasize through contrast the variations in susceptibility of infants of different age periods to the various infecting agents.

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[1] Die Lumbalpunktion. Ergebnisse der inneren Medizin und Kinderheilk., 1909, Bd. iii, S. 100.

[2] Prag. med. Wchnschr., 1907.

[3] Observations on 300 Cases of Acute Meningitis in Infants and Young Children, Am. Jour. Dis. of Child., 1911, i, 26.

Review of the Literature. The earliest cases of meningitis which I found in the literature were reported by Sherer (1895[5]). He reported 3 cases in infants from eight days to six weeks old. One of these cases was a weak, jaundiced infant nine days old, suffering from vomiting, diarrhea and rapid loss in weight. It died, with definite meningeal symptoms, nine days later. At the autopsy considerable pus was found covering the convexity and the base of the brain. A bilateral suppurative otitis media was present. The bacteriological report was colimeningitis. Two other cases of infants, eight days and six weeks old respectively, occurred in the same hospital during a period of a little over a year. All presented the same type of lesions: fibrinopurulent colimeningitis and bilateral otitis media. Sherer considers his 3 cases as a small epidemic, due to overcrowding in the hospital. He thinks that the infection originated through the contaminated water used in bathing the infants. The infected material, lodging in the external ear, started the otitis media. From this location the infection spread to the brain. It is also possible for the infection to have gained entrance through the mouth and from there to have been carried through the lymphatics or, more probably, through the Eustachian tubes to the middle ears and then to the brain.

Hinsdale(1899[6]) describes a case of meningitis in an infant, three days old, caused by Bacillus coli immobile. The mother had suffered with an acute metritis following a miscarriage two years previously. During and after the expulsion of the placenta, large quantities of pus escaped from the uterus. The infant began losing in weight and on the third day the emaciation was quite marked. Nystagmus and retraction of the head were present from birth. Convulsions appeared on the fourth day. The umbilical cord had a fetid odor. The infant would not nurse and died on the thirteenth day. At autopsy an abscess was present in the left frontal lobe of the brain in addition to the exudate on the surface. There was an induration of the abdominal wall around the navel. The infection in this case probably arose from the previous endometritis. The process extended through the placenta to the fetus.

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[4] Meningitis in Infancy, Illinois Med. Jour., 1913, No. 3, xxiii, 299.

[5] Ein Beitrag zur Aetologie der Leptomeningitis Purulenta bei Säuglingen, Jahrbuch f. Kinderheilk., 1895, Bd. xxxix, S. 1.

[6] Purulent Encephalitis and Cerebral Abscess in the Newborn Due to Infection from the Umbilicus, Am. Jour. Med. Sc., 1899, cxviii, 280.

Scheib(1900[7]) cites a case of an eight-day-old infant who died with the diagnosis of general debility. At autopsy a purulent meningitis was found, caused by the Bacillus lactis aërogenes. This organism is considered a frequent cause of meningitis in infancy, but this case is the only one I found recorded.

Goldreich(1902[8]) cites an interesting case of antenatal infection with Bacillus coli. Convulsions, together with cyanosis, jaundice, diarrhea, rigidity of neck and irregular respirations, appeared in the infant on the first day of its life. It died on the second day. The principal finding at autopsy was a fibrinopurulent meningitis. A purulent pleuritis and acute bronchitis were also present. It is hardly conceivable that the infection in this case did not happen until after birth. The author suggests that the bacteria might have gained entrance into the uterus during labor, which in this case lasted three days. On the second day the amniotic sac ruptured, opening an avenue for the entrance of germs. Then the fetus aspirated some of the contaminated fluid through premature respirations. From the lungs the infection was carried to the brain by the blood. Such a presumption helps to explain the bronchitis and pleuritis as well as the meningitis found at autopsy. This is probably the youngest case of suppurative meningitis reported in the literature.

Benfey (1907[9]) cites a case of Bacillus pyocyaneus meningitis for the purpose of proving the pathogenicity of this organism. He reports a case of a seven-day-old infant who developed meningeal symptoms and died on the eighth day. The navel was distinctly inflamed and the author believes that it was the portal of infection. At autopsy a pure culture of Bacillus pyocyaneus was recovered both from the purulent exudate on the brain and from the blood.

Noeggerath's case (1907[10]) was in a female infant, sixteen days old, who was well up to the eleventh day. She then developed abdominal cramps and symptoms of meningitis. A purulent exudate was present on the umbilicus. In the eighth week a double otitis media appeared. An organism was isolated which seemed to correspond to Bacillus coli, but had properties also of Bacillus lactis aërogenes. It was encapsulated and quite motile. It was identified as Bacillus coli immobilis capsulatus. Stern[11] and Conradi and

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[7] Prag. med. Wchnschr., 1900, S. 169.

[8] Meningitis beim Neugebornen, Jahrbuch f. Kinderheilk., 1902, Bd. lvi, S. 808.

[9] Ueber Pyozyaneussepsis, Med. Klinik, 1907, Nr. 40, S. 1199.

[10] Bacillus Coli Immobilis Capsulatus bei Einem Falle von Eitriger Meningitis Cerebrospinalis, München. med. Wchnschr., 1907, Nr. 13, Bd. liv, S. 617.

[11] Zur Kenntnis der Pathogenen Wirkung des Koli beim Menschen, Deutsch. med. Wchnschr., 1893.

Bierast[12] speak of the many variations that may occur in the Bacillus coli when it becomes pathogenic. Noeggerath thought that the infection in this case was either through the navel or through the intestinal tract. An argument against the navel route was the microscopic finding of the umbilical vessels, which showed no evidences of any inflammation.

Holt (1911[13]) reports a series of 300 cases of meningitis in children under three years old, but from the data given I could classify only 11 of them as occurring in infants under three months old. Of these 11, 3 cases (a very large percentage) were due to Bacillus tuberculosis; 6 cases to staphylococcus and streptococcus and only 1 case to Bacillus coli. The last case presented meningeal symptoms only. It became sick when four weeks old and died in the sixth week. A pure growth of Bacillus coli was obtained from the purulent fluid removed from the spinal canal. The author cites an additional case of colimeningitis from his private practice. This was in a four-weeks-old infant who had previously suffered from a colon infection of the urinary tract.

Pearson's case (1912[14]) was in a female infant, three months old. At autopsy the Bacillus coli communis was isolated from the spinal fluid and from the purulent exudate enveloping the brain and filling the ventricles. There was also an acute suppurative otitis media present. The author holds this latter condition responsible for the meningitis.

Smith and Woodforde(1912[15]) report a case of colimeningitis in an infant somewhat older than the usual incidence of this infection. Their patient was five months old. The organisms isolated were very motile. The disease at first responded to autogenous vaccine therapy, but later the patient died. The urine in this case was sterile. The authors consider the meningitis as a primary infection.

A case of meningitis caused by the Bacillus mucosus capsulatus is reported by Bonhoff and Esch (1912[16]). The infant was born in asphyxia. A catheter was inserted into its trachea to initiate respiration. Small quantities of amniotic fluid and mucus escaped from the mouth when the infant started breathing. The first four days it was restless, whimpered a great deal and did not nurse well. Clonic contractions appeared in the face muscles and in the back muscles on the sixth day. The development of emaciation was rapid. Spinal fluid was removed and examined but no cultures were made. Death occurred on the fourteenth day. At autopsy there was pus present over the brain and in the right middle and internal ear. Culture from the pus on the brain and from the middle ear showed a pure growth of Friedländer's bacillus.

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[12] Kolle-Wassermann: Handbuch der Pathogenen Mikroorganismen, 2 Auflage, Bd. vi, S. 483.

[13] Loc. cit.

[14] A Case of Meningitis in Which the Bacillus Coli Communis was Obtained from the Cerebrospinal Fluid, Lancet, London, 1912, clxxxii, 722.

[15] Two Unusual Forms of Meningitis Occurring in Infancy, Lancet, London, 1913, clxxxiii, 236.

[16] Ueber Einem Fall von Meningitis purulenta beim Neugebornen, infolge rechtseitiger Mittleohrentzündung, Ztschr. f. Geburtsh. u. Gynäk., 1912, Bd. lxx, S. 886.

Lyall (1913[17]) gives a detailed report of a case of typhoid meningitis in a female infant four months old. She died six weeks after the onset of the symptoms. The postmortem examination showed a diffuse purulent meningitis with no lesions present characteristic of typhoid fever. The author thinks that the infection in this case took place through the intestinal tract.

Giese (1913[18]) reports the only case of influenzal meningitis in a young infant found in the literature, though some authors state that this type of infection is not uncommon.

Von Reus (1914[19]) reports a case of colimeningitis in a newborn infant. The case is cited by Michael,[20] with no data given.

Hermann (1915[21]) reports what he considers the only case of pneumococcic meningitis in the newborn. When thirteen days old the infant was taken sick. It died at the age of seventeen days. No meningeal symptoms were present at any time, yet the autopsy revealed a purulent meningitis as the only lesion present. Culture of the pus gave a pure growth of pneumococci. The author discusses the possible portals of infection. The umbilical route he considers rare. Premature respiration of the infant during difficult labor, he believes, may result in the aspiration of contaminated liquor amnii and thus start the infection. Infection may also occur through an injury to the skull during delivery. After birth the mouth, nose, throat, *intestinal tract*, ears, eyes, umbilicus or urinary tract may be the portals of entry. He states that the contaminated water of the bath-tub is a frequent source of infection, and thus agrees with Noeggerath[22] and Sherer.[23]

Michael (1916[24]) recovered the Bacillus coli communis from the spinal fluid of a five-months-old infant. It died two weeks later. The organism was very motile. The exudate was described as a thick brownish pus, a characteristic mentioned by a number of authors.

A valuable contribution to meningitis in infants is made by Koplik (1916[25]). He reports, with some detail, his findings in 12 of his own cases. Four of his cases were due to streptococcus; 3 to pneumococcus; 4 to meningococcus; 1 to Bacillus coli. One case of the streptococcus, 1 of the meningococcus and the case of Bacillus coli meningitis showed symptoms within a few days after birth.

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[17] Meningitis in an Infant Caused by the Typhoid Bacillus, Jour. Med. Res., 1913, No. 4, xxvii, 457.

[18] Ugeskrift for Laeger, Copenhagen, 1913, No. 16 (Abs. in Jour. Am. Med. Assn.).

[19] Cited by Michael (20).

[20] Recovery of the Colon Bacillus from the Spinal Fluid of a Five-months-old Infant, Arch. of Ped., 1916, xxxiii, 280.

[21] Meningitis in the Newborn, with Report of a Case, Arch. of Ped., 1915, No. 8, xxxii, 583.

[22] Loc. cit.

[23] Loc. cit.

[24] Loc. cit.

[25] Meningitis of the Newborn and in Infants under Three Months of Age, Arch. of Ped., 1916, xxxiii, 481.

| Case No. | Author. | Year. | Age. | Type of bacterium. | Probable portal of entry. |
|---|---|---|---|---|---|
| 1 | Sherer | 1895 | Newborn | B. coli | Otitis media. |
| 2 | Sherer | 1895 | Newborn | B. coli | Otitis media. |
| 3 | Sherer | 1895 | 6 weeks | B. coli | Otitis media. |
| 4 | Hinsdale | 1899 | Newborn | B. coli | Placenta. |
| 5 | Scheib | 1900 | Newborn | B. lactis aërogenes | |
| 6 | Goldreich | 1902 | Newborn | B. coli | Lungs. |
| 7 | Benfey | 1907 | Newborn | B. pyocyaneus | Umbilicus. |
| 8 | Noeggerath | 1907 | 16 days | B. coli | Intestinal. |
| 9 | Holt | 1911 | 3 months | B. tuberculosis | |
| 10 | Holt | 1911 | 3 months | B. tuberculosis | |
| 11 | Holt | 1911 | 3 months | B. tuberculosis | |
| 12 | Holt | 1911 | Newborn | Staphylococcus and streptococcus | Spina bifida. |
| 13 | Holt | 1911 | Newborn | Staphylococcus and streptococcus | Spina bifida. |
| 14 | Holt | 1911 | Newborn | Staphylococcus and streptococcus | Spina bifida. |
| 15 | Holt | 1911 | Newborn | Staphylococcus and streptococcus | Spina bifida. |
| 16 | Holt | 1911 | Newborn | Staphylococcus and streptococcus | |
| 17 | Holt | 1911 | Newborn | Staphylococcus and streptococcus | |
| 18 | Holt | 1911 | 4 weeks | B. coli | |
| 19 | Holt | 1911 | 4 weeks | B. coli | |
| 20 | Pearson | 1912 | 3 months | B. coli | Suppurative otitis media. |
| 21 | Smith and Woodforde | 1912 | 5 months | B. coli | Hematogenous. |
| 22 | Bonhoff and Esch | 1912 | Newborn | B. mucosus capsulatus | Eustachian tube. |
| 23 | Lyall | 1913 | 4 months | B. typhosus | Intestinal. |
| 24 | Giese | 1913 | ? | B. influenzæ | |
| 25 | v. Reus | 1914 | Newborn | B. coli | Hematogenous. |
| 26 | Herrman | 1915 | Newborn | Pneumococcus | |
| 27 | Michael | 1916 | 5 months | B. coli | Hematogenous. |
| 28 | Koplik | 1916 | Newborn | Meningococcus | |
| 29 | Koplik | 1916 | 6 weeks | Meningococcus | |
| 30 | Koplik | 1916 | 3 months | Meningococcus | |
| 31 | Koplik | 1916 | 3 months | Meningococcus | |
| 32 | Koplik | 1916 | Newborn | B. coli | Circumcision. |
| 33 | Koplik | 1916 | 6 weeks | Streptococcus | |
| 34 | Koplik | 1916 | 7 weeks | Streptococcus | |
| 35 | Koplik | 1916 | 3 months | Streptococcus | |
| 36 | Koplik | 1916 | 6 weeks | Streptococcus | |
| 37 | Koplik | 1916 | Newborn | Pneumococcus | |
| 38 | Koplik | 1916 | 8 weeks | Pneumococcus | |
| 39 | Koplik | 1916 | 3 months | Pneumococcus | |
| 40 | White | 1917 | 29 days | Meningococcus | |
| 41 | White | 1917 | 39 days | Catarrhalis (?) | |
| 42 | Barron | 1917 | Newborn | B. coli | Mouth (?) |

The above table gives in tabulated form all the cases within the ages under discussion which were found in the literature.

The last-mentioned case occurred in a male infant who was circumcised on the eighth day. Three days later a colipyelitis set in, which was followed a week afterward by colimeningitis. The patient died at the age of sixteen months from asthenia and necrosis of both eyes, associated with marked hydrocephalus. The author emphasizes the fact that infections may occur through abrasions in the buccal mucous membranes produced by the accoucheur.

White (1917[26]) reports 2 cases in infants twenty-nine and thirty-nine days old respectively. The meningitis in the first case was caused by the meningococcus. From the second case an organism was isolated which was thought to be the Micrococcus catarrhalis. However, this diagnosis is questionable.

The following table gives the classification of the above forty-two cases according to ages:

| | |
|---|---|
| Newborn | 19 |
| Two weeks to one month | 4 |
| One month to two months | 7 |
| Two months to three months | 8 |
| Three months to five months | 3 |
| Exact age uncertain | 1 |
| Total | 42 |

Subtracting the 3 cases of three to five months old leaves 39 cases under three months.

The following table shows the classification of the 19 newborn cases according to etiology:

| | |
|---|---|
| Bacillus coli | 7 |
| Streptococcus and staphylococcus | 6 |
| Pneumococcus | 2 |
| Meningococcus | 1 |
| Bacillus mucosus capsulatus | 1 |
| Bacillus lactis aërogenes | 1 |
| Bacillus pyocyaneus | 1 |
| Total | 19 |

The following table shows the classification of the 42 cases according to etiology:

| | |
|---|---|
| Bacillus coli | 14 |
| Streptococcus and staphylococcus | 10 |
| Meningococcus | 5 |
| Pneumococcus | 4 |
| Tuberculosis | 3 |
| Bacillus mucosus capsulatus | 1 |
| Bacillus influenzæ | 1 |
| Bacillus typhosus | 1 |
| Bacillus lactis aërogenes | 1 |
| Bacillus pyocyaneus | 1 |
| Bacillus catarrhalis (?) | 1 |
| Total | 42 |

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[26] Case Reports of Infectious Meningitis Occurring in Babies Twenty-nine and Thirty-nine Days Old Respectively, Arch. of Ped., 1917, xxxiv, 372.

The above shows that the Bacillus coli causes practically 33 per cent. of all cases of meningitis in infants under three months. (Three cases in the table are over three months old.) Of this 33 per cent. (14 cases), one-half (7 cases) occurred in the newborn. This is strongly in contrast with the etiology of non-epidemic meningitis in infants of the second year of life, as is well brought out by the statistics of Holt[27] and others.

Case Report.—My own case is that of a male infant, eleven days old, who was delivered in breech presentation. The mother is a hard-working woman, aged forty-four years, who has had eight pregnancies, including the last one. Six children are living and well. During the latter months of the last pregnancy the mother experienced pain in the epigastrium which was of the character of real labor pains during the last two weeks before delivery. She noticed that her abdomen was excessively enlarged. The childbirth, she thinks, was about one month premature. The confinement was in the home, with practically no medical attention up to the second stage of labor. That there was a condition of hydramnios became evident when, during the second stage of labor, more than 1 gallon of amniotic fluid escaped. The delivery of the head was assisted by the insertion of a finger into the mouth of the fetus and then using a moderate amount of traction. The infant was cyanotic at birth, and it required considerable effort to initiate respiration.

The infant had a whimpering cry and at no time nursed well. A slight right-sided paralysis made its appearance on the third day. The right hand appeared puffy and edematous. On the tenth day there was a bilateral swelling of the eyelids. Several slight convulsions were noticed on the morning of the eleventh day. The lips turned cyanotic. The child died on the same day.

*Autopsy Protocol.* I performed the autopsy fourteen hours postmortem. I shall indicate only the important findings.

The body is fairly well developed, somewhat undernourished. The skin and conjunctivæ are slightly jaundiced. There is a depression 3 mm. deep over the left parietal eminence. This measures 2 x 4 cm. The skin over it is not abraded. The umbilicus has dropped off; the navel is dry and appears healthy. The right lung shows petechial hemorrhages beneath the pleura and in other portions of the parenchyma. The gastro-intestinal tract shows no lesions and appears entirely normal. Both adrenals are large, distended and of a bluish-red color. On section, large hemorrhages are found involving the medullæ, which extend more than half the distance into the cortices.

In removing the scalp no lesions are visible over the depressed area, nor is there any evidence of injury to the brain after the calvarium and dura mater are removed. The brain is found covered with a layer of brownish fibrinopurulent exudate. This is most marked over the left parietal region. The base of the skull and middle ears are free from lesions.

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[27] Loc. cit.

Smears from the exudate show disintegrated pus cells and immense numbers of extra- and intracellular Gram-negative, short, plump bacilli.

Microscopically the lungs show small areas of congestion and hemorrhage. The heart and spleen are normal. The liver shows a mild fatty metamorphosis. Many of the cells are swollen and their nuclei are vesicular. The adrenals show hemorrhages into the medullæ, with only an occasional parenchymal cell visible. The inner portions of the cortices are torn; the cords of cortical cells are separated by areas of hemorrhage. The kidneys show intense congestion of the glomerular tufts. The thymus shows moderate "accidental" involution.

The brain parenchyma is normal. The pial vessels are greatly dilated. The arachnoid space is filled with an exudate of fibrin, a few red blood cells and masses of leukocytes, nearly all of which are polymorphonuclears. In some areas the exudate consists almost wholly of fibrin and red blood cells.

*Bacteriological Studies.* Cultures were made from the exudate and from the heart's blood. This, together with the animal inoculations, was performed by Dr. Arthur T. Henrici. I am indebted to him for the data of the bacteriological studies.

The report states that the smears from the pus show a very short, Gram-negative coccobacillus. Some of the bacilli stain more deeply at the poles than in the middle and give the appearance of diplococci.

The cultures from the heart's blood showed no growth. The first cultures from the pus showed a short, slender bacillus, with a few coccoid and irregular forms. Some forms possessed club-shaped extremities. The subcultures and all subsequent cultures gave a fairly uniform bacillus typical of Bacillus coli. In hanging-drop preparations all of the cultures showed a very active, typhoid-like motility.

The cultural characteristics of this organism on the different media are as follows:

Agar: spreading, moist, grayish growth.

Broth: uniform turbidity.

Gelatin stab: filiform growth; no liquefaction.

Litmus milk: first culture acid in four days; no coagulation. Subsequent cultures: acid in two days; coagulation in seven days; very little whey expressed.

Peptone solution: indol in four days.

Sugars: dextrose, acid and gas in twenty-four hours.
maltose, acid and gas in twenty-four hours.
mannite, acid and gas in twenty-four hours.
lactose, acid in forty-eight hours; gas in four days.
saccharose, no acid and no gas.

(Gas formula $= \frac{CO_2}{H_2} = \frac{1}{2}$.)

In all the subcultures the lactose fermented a day or two later than did the other sugars.

From the above it will be seen that the organism recovered was definitely the Bacillus coli communis, but the marked motility and the tardiness in its action on lactose at first suggested the Bacillus paratyphosus.

Inoculations into rabbits and guinea-pigs, either intravenous or intraperitoneal, killed the animals in from twelve to forty-eight hours. The organism was recovered from the blood in each case. No immunity reactions were performed.

COMMENT. Conradi and Bierast[28] speak of the variations in size, shape and motility in Bacillus coli. They also mention that some strains are slow in fermenting lactose. Actual mutations are thought to occur, and these strains are sometimes designated as Bact. coli mutabile. Most of the strains of Bacillus coli isolated from the meningitis cases here reported were actively motile.

The susceptibility of infants to Bacillus coli infections has long been noted. Beitzke[29] states that Bacillus coli is the principal causative agent of pyelitis, pyelocystitis and pyelonephritis. It also sometimes produces intestinal diseases, general sepsis and meningitis. Yet in the adult this organism is almost entirely a saprophyte, being a constant inhabitant of the intestinal tract, and rarely does it become pathogenic. But since the protective powers of the infant are very feeble the bacillus occasionally gets a foothold and becomes pathogenic. Halban and Landsteiner state that infants possess a very low capacity for the manufacture of protective substances. Artificially-fed infants are said to be even more susceptible to infections than breast-fed ones, because the mother's milk contains immune bodies which are utilized by the infant.

La Fetra[30] does not believe that infants are born septic very often. Such instances, he thinks, are indeed rare. He also doubts that infection is common through the aspiration of contaminated liquor amnii, as claimed by Sherer[31] and others. Bonhoff and Esch,[32] on the other hand, claim that in their case the infecion was through the Eustachian tube, brought about by the aspiration of liquor amnii during premature breathing in intra-uterine life. The fluid and mucus which escaped from the mouth of the infant at the beginning of respiration certainly warrants such a contention. The introduction of the catheter may also have carried the infection. Aschoff[33] has found in his careful studies that the passage of liquor amnii up the Eustachian tubes during premature respiration is fairly common. He, however, believes that the inflammation resulting from it is not due to infection introduced but only to the fluid itself acting as a foreign body. Rasch[34] studied the postmortem findings relative to middle-ear diseases in 82 autopsies on infants up to two years old. He found that fully 75 per cent. had either unilateral or bilateral middle-ear involvement, many cases of which were purulent in character. Several cases had meningitis associated with it. Such a finding strongly suggests that meningitis must be not infrequently secondary to middle-ear infections. If this finding be correlated with the finding of Aschoff,[35] that there is a *locus minoris resistentiæ* in the middle ears due to the presence of aspirated amniotic fluid, then the sequence of events leading up to many cases of meningitis becomes almost self-evident.

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[28] Loc. cit.

[29] In Bruning und Schwalbe's Handbuch der Allgemeine Pathologie, Bd. i, S. 252.

[30] Accidents and Diseases of Early Weeks, Arch. of Ped., 1916, xxxiii, 401.

[31] Loc. cit.

[32] Loc. cit.

[33] Ztschr. f. Ohrenheilk., Bd. xxxi, S. 295.

But La Fetra[36] believes that the umbilicus is the most important route. Also that the skin and mucous membranes are frequent avenues because these tissues are comparatively undeveloped at birth. The intestinal mucous membrane, for example, is undoubtedly pervious to bacterial invasion without any evident lesions. The cases of bovine tuberculosis in infants and young children without intestinal tuberculosis certainly corroborate such a concept. As to the mucous membrane of the mouth, as a portal of entry, one can get little definite proof. In my case I believe that the infection occurred through the introduction of the accoucheur's finger during delivery. Koplik[37] calls attention to this method of introducing infection. A woman who has not been properly prepared for delivery will have the vulva more or less contaminated with fecal material. The introduction of a finger into the mouth of the infant in the process of delivery might create just the proper conditions for infection: the introduction of the infectious agent and the abrasion of the covering epithelium, the mucous membrane.

There is some evidence in favor of the idea that the external auditory canal is also an important avenue for infection in the infant. Rasch[38] believes that of the two avenues of infection of the middle ear, that of the Eustachian tube and of the external auditory canal, the latter is the more important one.

GENERAL CONCLUSIONS. 1. Meningitis in the newborn and in early infancy is a rare disease.

2. The important place that the Bacillus tuberculosis holds in the meningitis of later infancy the Bacillus coli occupies in the early months of infant life.

3. Pathogenic strains of Bacillus coli may show marked variations in form, in rates of fermentation and in motility.

4. The avenues of infection of the newborn have not been definitely established. Nevertheless, infection through the mouth

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[34] Ueber die Häuflichkeit und Bedeutung von Mittleohrentzünd bei Kleinen Kranken Kindern, Jahrbuch f. Kinderheilk., 1894, Bd. xxxvii, S. 319.

[35] Loc. cit.

[36] Loc. cit.

[37] Loc. cit.

[38] Loc. cit.

by means of fingers or instruments of the accoucheur must always be borne in mind and proper precautions taken accordingly.

5. It is reasonable to believe that some infections occur in the bath-tub through water that has become contaminated. The portals of entry of the bacteria may be either through the external auditory canals, the mucous membrane of the mouth, the Eustachian tubes or the intestinal tract.

6. The susceptibility of infants to infection with organisms, that are otherwise only slightly pathogenic, may be explained by the feebleness of antibody production during the early months of infancy. The greater resistance of breast-fed infants over the artificially-fed ones is probably due to the compensation of the passive immunization by the breast milk for the active immunization which is still deficient.

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# PATHOLOGY OF (MUSTARD?) GAS INHALATION.

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Introduction. The object of this paper is to offer a description of the gross and microscopic pathology produced by the inhalation of (mustard[1]) gas. Of the 37 cases included in this study, 35, according to the field medical cards, were due to mustard gas, 1 to mustard gas and phosgene and 1 to phosgene alone. In 34 cases the action of the gas on the respiratory tract was the main factor in causing death. Two died as a result of extensive skin burns, and 1 of cellulitis of the face and neck following burns in this region. It is evident that though the skin and eyes suffer from contact with this agent, and though death may result from injury in these areas, inhalation and the resulting pathology in the respiratory tract is the important factor in cases which reached this center.

The respiratory tract is attacked from the tip of the epiglottis to the terminal bronchioles and air vesicles. The effects seen here are due (1) to the intense irritation and escharotic action of the gas, and (2) to secondary infection, which promptly occurs. As seen in the autopsy room, more or less of the entire mucosa over this region is covered by a fibrinopurulent exudate, a false membrane, from dirty gray to yellowish or greenish-yellow in color depending somewhat on the amount of suppuration and blood pigments present. In many cases it covers the entire area from the tip of the epiglottis downward; in others it is in patches; and in relatively few does it cover the wall of the lower part of the pharynx.

The appearance of this membrane, both grossly and microscopically is very similar to that usually seen in diphtheria. In addition, the clinical features are not unlike those in the laryngeal type of this disease: hoarseness, temperature and frequently a relatively rapid pulse. Also, throat cultures often showed that many of them harbored the typical organisms. As a result, it not infrequently happened that perplexity arose in the differential diagnosis and several times an erroneous diagnosis of diphtheria, or *vice versa,* was made.

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[1] The term "mustard" gas is used because of the statements found on the clinical records, together with the character of the lesions which corresponds to that described in the literature.

In nearly all cases the larynx is attacked. Here the false membrane extends down into all the irregularities of this region. The reaction is usually accompanied by more or less edema surrounding the base of the epiglottis, the aryepiglottic folds and the false vocal cords. When the membrane is removed a deeply injected, hemorrhagic surface is revealed, usually showing shallow ulcerations. These are particularly likely to occur along the prominent and more rigid portions, such as the tip of the epiglottis and the edges of the vocal cords.

In the trachea and larger bronchi, much the same pathology exists. There is the same sort of membrane with the injection, hemorrhage and ulceration of the underlying tissues. Here also the ulceration tends to occur on the more prominent portions, such as the mucosa over the cartilaginous rings.

In cases of longer standing the membranous exudate is less in evidence and the ulceration of the tracheal and bronchial walls is more marked. In one case (Fig. 1) the mucosa of the lower half of the trachea and of the bronchi in the region of the bifurcation was of a greenish hue and literally riddled with ulcers, one of which was 1 cm. in diameter and extended through the cartilaginous ring to the outer fibrous coat of the trachea.

The lungs are usually very voluminous, due to a marked emphysema, largely vesicular, but occasionally interstitial or bullous. Subpleural hemorrhages are frequent. There may or may not be a pleurisy depending upon the extent of the secondary infection present. There is commonly the nodular feel of a bronchopneumonia and occasionally the solidity of a massive consolidation. The cut surface is characteristic. In very mild or very early cases there may be only congestion, hemorrhages and edema of the parenchyma surrounding the bronchial tree, together with emphysema of the peripheral portions; but ordinarily the exudate described as occurring in the trachea and bronchi extends down to the finest radicles, which are not simply lined with the membrane but actually plugged. These plugs project above the cut surfaces and may be squeezed out, much like comedones. About many of these bronchioles there is a larger or smaller area of infiltration, resembling bronchopneumonia, raised, grayish or dark red (if hemorrhagic) and dry and granular in appearance. The hemorrhagic areas are prominent. Outside the pneumonic area is the vesicular emphysema.

In the older and more advanced cases necrosis is frequently present in the consolidated areas, and this becomes the more prominent feature. This occurs particularly nearer the hilus where the pneumonic areas are larger, confluent and massive, but may also be seen in the centers of the smaller discrete areas. The necrosis gives rise to abscess cavities, the larger of which have ragged, irregular walls and are filled with thin, grayish, granular, purulent and necrotic material.

Microscopically the pathology is very interesting. In the upper respiratory tract the membranous exudate lining the lumen is exactly similar to that seen in diphtheria, consisting of masses of fibrin mixed with occasional leukocytes, cellular debris and plasma. On the free surface of this exudate numerous clumps of bacteria are seen, principally diplococci. There is often no differentiation between the exudate and the mucosa of the trachea or bronchi. The epithelium is nearly everywhere absent. In a few places degenerating or necrotic cells can still be made out. The submucous tissue is infiltrated with leukocytes and is definitely edematous. There is not quite as extensive hyalinization of the fibrin, however, as one finds in a diphtherial membrane.

This type of exudate extends down into the bronchioles, where at times the lumina are completely plugged. The exudate is frequently seen attached at only one point, and here the membrane blends with the wall of the bronchiole so intimately that no differentiation is possible. The wall is markedly infiltrated with leukocytes, and this condition extends into the contiguous alveoli. In the unattached portions of the bronchial wall the epithelium may still persist. It is at the point of attachment of the fibrinopurulent membrane that the ulceration and necrosis apparently begins, and from there it extends into the deeper portions of the lung parenchyma, resulting in the formation of abscesses of the lung. In the upper portion of the tract, namely, the prominent edges of the epiglottis, vocal cords and cartilaginous rings, similar ulcerations are encountered. These ulcerations may perhaps be explained by the fact that the portions protruding into the lumen are attacked by larger quantities or by more concentrated mixtures of the poisonous gases. It is not impossible that minute droplets of the liquid gas itself are inhaled. In the lower portion of the tract the direction of the air current may determine the site of greatest injury.

The histological picture varies, depending upon the rapidity of death after the injury and consequently on the dose and concentration of the inspired gas. In several cases that came to autopsy the dose was evidently overwhelming, resulting in death from the escharotic and irritating action alone. In these rapid cases the prominent features are the edema, the profound injection of the bloodvessels and the hemorrhage. The edema generally differs from ordinary pulmonary edema in that the fluid escaping into the alveoli is considerably more albuminous, so that when coagulated it greatly resembles colloid. The fluid not only fills the vesicles around the bronchioles, but often is seen separating the cellular structure of the alveolar walls. In areas farther away from the bronchioles the albuminous fluid is deposited principally in the neighborhood of the alveolar walls, leaving clear spaces in the centers of the vesicles. Some areas show marked fibrin deposits lining the walls of the alveoli in a manner similar to the exudate seen in the bronchioles or even in the bronchi. The only difference noticeable is that there is a relative absence of leukocytic infiltration. In the centers of these alveoli there may be only a thin serum deposited or a very fine fibrinous network; sometimes the space is entirely free from exudate. In older stages the fibrin becomes hyalinized and shows an infiltration of leukocytes and the presence of degenerating desquamated epithelial cells.

Such a picture as above described helps explain the very marked dyspnea and cyanosis so frequently encountered in these cases clinically. Both the albuminous fluid around the walls of the air vesicles and the lining by dense fibrinous masses must produce a very profound interference with the normal interchange of gases in the alveoli.

Another marked feature is the injection of the bloodvessels. In many of the bronchial walls the enormously dilated capillaries present a picture of a capillary hemangioma. Also the extensive hemorrhage, which is so commonly seen in the alveoli surrounding the bronchioles, gives a picture, with the low power of the microscope, which resembles a cavernous hemangioma of the liver. The capillaries of the alveolar walls are dilated and tortuous. It requires very little imagination to visualize the processes by which the extensive hemorrhages occur.

The histology of the peripheral portions of the lungs shows little except vesicular emphysema. In later stages, with advanced pneumonic involvement, pleurisy may be present.

In the older cases, that is, in cases which die of the secondary infection rather than from the direct effects of the gas, there is a picture of bronchopneumonia. But even here the marked edema and hemorrhage, along with the peculiar deposits of fibrin, make the diagnoses fairly easy. Areas of necrosis are now present. These apparently begin in the walls of the bronchioles and extend centripetally, forming occasionally large abscesses filled with pus and necrotic debris. Around these necrotic areas the alveolar walls often show thickening due to fibrosis and infiltration, with round cells and polymorphonuclears. The alveoli contain dense masses of precipitated serum or fibrin in various stages of organization. Some show only a slight infiltration with leukocytes; others show fibroblasts from the alveolar walls invading the masses; still others show fairly well-advanced organization of the exudate. These areas are generally small and surround the bronchioles in the regions where the abscesses are apparent. The picture is unlike the ordinary types of organization pneumonias, in that the areas of necrosis and the abscesses are more common. Some of the bronchioles also show organization of the exudate, so that if the case should go on to healing the bronchioles would probably become organized into fibrous cords.

No changes were evident in the bronchial walls that could be taken for bronchiectases. However, the various lesions seen in the bronchi and bronchioles might lead to great weakening and dilatation if the patient should go on to recovery.

In one of the cases studied the condition had gone on for several months and yet no bronchiectases were apparent in the lungs. In the cases of influenza-pneumonias studied in this center, bronchiectatic changes were relatively common and were apparent in lesions of much shorter duration. It is therefore quite likely that the lung pathology following influenza is more susceptible to the development of bronchiectasis than that following gas inhalation.

The pathogenesis of these lesions cannot be completely worked out from the material obtained at this center, because no cases came to autopsy under four or five days after exposure. However, by correlating the findings, and by making a composite picture of all the lesions encountered in the various stages of the disease, one might visualize the processes of reaction about as follows:

As the irritating gas is inspired, some of the exposed cells may become immediately necrosed. The capillaries nearest the surfaces become engorged. This is true also in the smallest bronchioles and perhaps in the alveoli themselves. The irritation causes an exudate consisting of a moderate quantity of heavy albuminous fluid in contradistinction to the huge quantities of thin fluid poured out in cases of phosgene inhalation. Later, cellular elements appear in the exudate along with desquamated epithelial cells. Some of the fibrinogen is converted into fibrin, which forms a lining not only along the upper tube of the respiratory tract but also may extend down into the terminal bronchioles and air cells. Depending upon the amount of irritation, the exudate, which is now fibrinopurulent in character, may increase in thickness until it may produce a layer 3 or 4 mm. in depth in the larynx, trachea and bronchi. There is at the same time a marked edema and congestion of the loose subepithelial cellular tissues. In proportion to the amount of congestion and the extent of the necrosis produced will be the extent of the ulceration along the larynx, trachea and bronchi and the hemorrhages in the lung parenchyma. The amount of exudate in the smaller bronchioles may become so abundant as to completely plug the lumina. This obstruction of some of the bronchioles will result in the compensatory emphysema of other portions of the lung.

| Case No. | Hosp. No. | Days after gassing. | Skin and eyes. | Larynx. | Trachea. | Bronchi. | Bronchioles. | Lung. | Clinical data. | Cause of death. |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 25–27 | 18 | Extensive burns; severe conjunctivitis | False membrane; hemorrhage; ulceration | False membrane; hemorrhage; ulceration | False membrane; hemorrhage; ulceration | Plugged with thick exudate | Small patches of consolidation | ....... | Skin, burns of, extensive. |
| 2 | 25–32 | 6 | Severe second degree burn of face, neck, buttocks, thighs; severe conjunctivitis | Heavy false membrane; surface ulcerated; edema of glottis | Heavy false membrane; lower part hemorrhagic | Thin fibrinopurulent membrane | Many occluded by plugs of fibrinopurulent membrane | Edema; small patches of consolidation | Cyanosis; severe cough; pain in chest; albuminuria | Acute fibrinopurulent tracheobronchitis. |
| 3 | 25–38 | 6 | Skin mild, not extensive burns; eyes inflamed | Purulent exudate; hemorrhagic | Injected; thin fibrinopurulent exudate | Thick fibrinopurulent exudate | Many completely plugged with thick exudate | Marked edema; moderate patches of consolidation | Fever; eyes swollen; pain in chest; delirium | Acute hemorrhagic and purulent tracheobronchitis. |
| 4 | 25–39 | 6 | Mild skin burns | Thick purulent exudate; hemorrhage; ulceration | Thick purulent exudate; hemorrhage; ulceration | Thick purulent exudate; hemorrhage; ulceration | Many completely plugged with thick exudate | Emphysematous; small nodules of consolidation | Fever; dyspnea | Acute ulcerative and suppurative tracheobronchitis. |
| 5 | 25–40 | 20 | ....... | Congested; thin layer of purulent exudate | ....... | Congestion; fibrinopurulent exudate | Congested fibrinopurulent plugs | Small patches of bronchopneumonia; edema | Temperature; swelling of eyes; bronchitis and pulmonary edema | Acute suppurative laryngitis and bronchitis; bronchopneumonia. |
| 6 | 25–45 | 29 | Large superficial skin burns; eyes congested and lids ulcerated | Injected; thickened; yellow thick purulent exudate; ulceration | Injected mucosa thickened | Fibrinopurulent membrane | ....... | Diffuse bilateral bronchopneumonia; edema; emphysema | Cyanosis; dyspnea; conjunctivitis; signs of bronchopneumonia | Acute suppurative tracheobronchitis and ulcerative laryngitis; bronchopneumonia. |
| 7 | 25–48 | 17 | Severe burns of genitals; eyes acutely inflamed | Ulceration; hemorrhage | Thick fibrinopurulent membrane; hemorrhage; ulceration | Thick fibrinopurulent membrane; hemorrhage; ulceration | ....... | Diffuse bronchopneumonia; emphysema marked | Fever; cyanosis and dyspnea | Acute suppurative and ulcerative tracheobronchitis; bronchopneumonia. |
| 8 | 25–50 | 12 | Severe burns of face; severe conjunctivitis | Congestion; hemorrhage; thin yellow fibrinopurulent exudate | ....... | ....... | ....... | Bronchopneumonia, diffuse; pulmonary necrosis; edema marked | Profuse expectoration; diffuse bronchitis; membrane on the soft palate | Bronchopneumonia and acute purulent tracheobronchitis. |
| 9 | 25–56 | 10 | Severe conjunctivitis | ....... | Thin yellow exudate; hemorrhage; ulceration | Thin yellow exudate; hemorrhage; ulceration | ....... | Bronchopneumonia, diffuse emphysema and edema marked | Fever; cyanosis; dyspnea; diffuse bronchitis | Acute suppurative tracheobronchitis and bronchopneumonia. |
| 10 | 26–16 | 3 | Mild skin burns; severe hemorrhagic conjunctivitis | Hemorrhage; ulceration; purulent exudate; edema of glottis | Hemorrhage; ulceration; purulent exudate | Hemorrhage; fibrinopurulent exudate | Many completely plugged with thick exudate | Early bronchopneumonia; marked emphysema | Coma; dyspnea; cyanosis; temperature and rapid pulse | Acute hemorrhagic and suppurative tracheobronchitis and edema of glottis; bronchopneumonia. |

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|---|---|---|---|---|---|---|---|---|---|
| 11 | 26-18 | 4 | Burn of genitalia; severe conjunctivitis | Hemorrhage; edema; plastic exudate | Hemorrhage; edema; plastic exudate | Hemorrhage; edema; plastic exudate | Many completely plugged with exudate | Early bronchopneumonia; marked emphysema | Cyanosis; dyspnea | Acute hemorrhagic and suppurative tracheobronchitis; bronchopneumonia. |
| 12 | 26-26 | 13 | ....... | ....... | ....... | Left has plastic exudate | ....... | ....... | Temperature; dyspnea; albumin and casts | Acute fibrinous bronchitis and edema of lungs. |
| 13 | 26-30 | 17 | Severe second degree burns; severe conjunctivitis | ....... | ....... | Congested; moderate purulent exudate | Congested | ....... | Burns of skin only | Severe skin burns. |
| 14 | 26 44 | 5 | Severe conjunctivitis | Fibrinopurulent exudate | Congestion and fibrinopurulent exudate | Congestion and fibrinopurulent exudate | Purulent exudate | Massive bronchopneumonia | Cyanosis; dyspnea | Bronchopneumonia; suppurative bronchitis and tracheitis. |
| 15 | 26-46 | 6 | Congestion and hemorrhage in the conjunctivæ | Fibrinopurulent exudate | Fibrinopurulent exudate | Fibrinopurulent exudate | Many plugged with heavy fibrinopurulent exudate | Moderate bronchopneumonia; edema | Dyspnea; conjunctivitis | Acute fibrinopurulent tracheobronchitis; bronchopneumonia. |
| 16 | 26-52 | 10 | Second degree burns of scrotum; severe conjunctivitis | Hemorrhage; ulceration; fibrinopurulent exudate | Hemorrhage; ulceration; fibrinopurulent exudate | Hemorrhage; ulceration; fibrinopurulent exudate | Purulent exudate | Bronchopneumonia | Fever; sore throat; signs of pneumonia | Acute suppurative ulcerated tracheobronchitis; bronchopneumonia. |
| 17 | 26-56 | 9 | Slight skin burns; marked acute conjunctivitis | Thick exudate; vocal cords edematous and ulcerated | Heavy fibrinopurulent exudate | Casts of bronchi in pseudomembrane | Plugged with thick exudate | Early bronchopneumonia | Dyspnea; cyanosis; fever; signs of pneumonia | Diphtheria; edema of glottis; acute suppurative and ulcerative tracheobronchitis; bronchopneumonia. |
| 18 | 26 59 | 25 | Moderate skin burns; ulceration of eyelids | Plastic exudate; ulceration | Marked fibrinopurulent exudate | Very marked fibrinopurulent exudate | Many completely plugged with thick exudate | Marked bronchopneumonia | Fever; dyspnea; cough | Acute ulcerative and membranous tracheobronchitis; bronchopneumonia. |
| 19 | 26-62 A. | 21 | Moderate skin burns; ulceration of lids | Fibrinopurulent pseudomembrane | Hemorrhage; ulceration; fibrinopurulent exudate | ....... | Pus | Advanced bronchopneumonia; necrosis (early) | ....... | Bronchopneumonia; ulcerative and membranous tracheobronchitis. |
| 20 | 26-74 | 17 | ....... | Fibrinopurulent pseudomembrane | Fibrinopurulent pseudomembrane | Pseudomembrane and pus | Many plugged with thick exudate | Early bronchopneumonia; edema; emphysema | Fever; hoarseness; cough; pain in chest; dyspnea | Acute suppurative tracheobronchitis; bronchopneumonia. |
| 21 | 26-1 | 11 | Severe skin burns of face and neck; eyes burned | ....... | ....... | ....... | ....... | ....... | ....... | Cellulitis of face and head following burns in these regions. |
| 22 | 49 32 | 7 | Severe skin burns; eyes injected | Thick fibrinopurulent exudate; ulceration; hemorrhage | Thick plastic exudate; ulceration; hemorrhage | Thick plastic exudate; ulceration; hemorrhage | Many plugged with thick exudate | Edema | Dyspnea; pain in chest; weak pulse | Acute fibrinopurulent tracheobronchitis. |
| 23 | 49-34 | 4 | Severe, not extensive burns; purulent conjunctivitis | Thick fibrinopurulent exudate; ulceration | Thick fibrinopurulent exudate; ulceration | Thick fibrinopurulent exudate; ulceration | Pus | Hemorrhage | Cough; dyspnea; cyanosis | Acute suppurative and ulcerative tracheobronchitis. |

| Case No. | Hosp. No. | Days after gassing. | Skin and eyes. | Larynx. | Trachea. | Bronchi. | Bronchioles. | Lung. | Clinical data. | Cause of death. |
|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 49–36 | 5 | Slight burns of face; eyes swollen | Greenish-yellow fibrinopurulent exudate | Greenish-yellow fibrinopurulent exudate | Greenish-yellow fibrinopurulent exudate | Plugged with similar exudate | Bronchopneumonia moderate; edema | Fever; delirium; eyes swollen | Acute suppurative and ulcerative tracheobronchitis; bronchopneumonia. |
| 25 | 49–51 | 7 | ....... | ....... | Fibrinopurulent pseudomembrane | ....... | Plugged with similar exudate | Bronchopneumonia; marked edema; emphysema | Fever | Acute fibrinopurulent tracheobronchitis and bronchopneumonia. |
| 26 | 49–67 | 25 | Mild burns of head, chest, arms; conjunctivitis | Fibrinopurulent exudate | Fibrinopurulent exudate | ....... | Many plugged with similar exudate | Bronchopneumonia; marked emphysema | ....... | Acute fibrinopurulent tracheobronchitis and bronchopneumonia. |
| 27 | 56–2 | 6 | ....... | ....... | Congested | Hemorrhagic | Hemorrhagic | Marked edema | ....... | Acute hemorrhagic bronchitis; edema of lungs. |
| 28 | 56–7 | ? | ....... | Fibrinopurulent membrane; congestion; edema | Fibrinopurulent membrane; congestion; edema | Congestion; edema | Congestion; edema | Very marked edema | ....... | Edema of glottis. |
| 29 | 56–14 | 16 | ....... | ....... | ....... | ....... | ....... | Bronchopneumonia | ....... | Bronchopneumonia. |
| 30 | 56–19 | 4 | Skin of face slightly burned; corneal opacity | Fibrinopurulent exudate; ulceration; hemorrhage | Fibrinopurulent exudate; ulceration; hemorrhage | ....... | Many plugged with similar membrane | Emphysema | ....... | Acute suppurative and ulcerative tracheobronchitis. |
| 31 | 56–30 | 18 | Few mild burns; conjunctivitis | Thin fibrinopurulent exudate and ulceration; edema of glottis | ....... | ....... | Many plugged with similar exudate | Early bronchopneumonia | Fever | Suppurative tracheobronchitis;bronchopneumonia. |
| 32 | 56–44 | 35 | Conjunctivitis | Pseudomembrane (gas and diphtheria); edema of glottis | Fibrinopurulent membrane | ....... | ....... | Hemorrhage | Fever; diphtheria | Septicemia; diphtheria; acute fibrinopurulent tracheobronchitis. |
| 33 | 56–57 | 34 | Conjunctivitis | Fibrinopurulent pseudomembrane | ....... | ....... | Pus | Massive bronchopneumonia | Violent cough, burning in chest; aphonia; nausea and vomiting | Acute fibrinopurulent tracheobronchitis; bronchopneumonia. |
| 34 | 56– | 70 | Acute conjunctivitis | ....... | Numerous deep ulcerations | ....... | Fibrinopurulent plugs and necrosis | Massive bronchopneumonia; abscess formation due to necrosis | ....... | Gangrenous and ulcerative tracheobronchitis; lung abscess; bronchopneumonia. |
| 35 | 19–1 | ? | Slight burns of face; congested conjunctivæ | ....... | ....... | Injected | Pus | Bronchopneumonia; emphysema marked | ....... | Bronchopneumonia. |

The upper respiratory tract is constantly exposed to infection, which will soon result in a superimposed inflammatory reaction, due to the bacterial invasion. This infective process will gradually descend into the bronchioles, owing to the lowering in the resistance of the tissues. It is at this time that polymorphonuclear infiltration may become very abundant and produce miliary or submiliary nodules, which finally may become confluent. Because the gas is in the greatest concentration in the upper portion of the respiratory tract the greatest pathological changes will be produced around the hilus. If the necrosis is very extensive, very profound ulcerations may develop in the trachea and larynx, which may almost penetrate the walls. In the lung parenchyma the necrosis will gradually develop into areas of hemorrhage and into abscess formation after the necrotic area becomes secondarily infected with bacteria.

If the patient receives severe burns in the respiratory tract but is still able to live for any length of time, for example, several months, then the resulting lesions will present the large areas of consolidation, pneumonic in character, through which will be scattered large or small areas of necrosis, with abscess formation. The attempt at healing will manifest itself by the definite evidences of organization of the exudate and the fibrosis, as previously described.

The following brief protocols are presented to illustrate the conditions found at autopsy in several cases of gas inhalation in which death occurred from five days to over two months after the injury. The first case is of a very early type while the last is an old and very advanced stage of the disease. Only the important data are given; the negative findings are omitted.

## CASE REPORTS.

CASE I.—Autopsy 26–16. E. B. P., Sergeant, Co. H, 306 Infantry, No. 1702538. Aged twenty-two years, white. Died September 28, 1918, at 6 A.M., at Base Hospital 26. Autopsied September 28, 1918, at 10 A.M. Capt. Moses Barron, M.C., pathologist.

"*Clinical Data.* This case is that of a young man admitted to Base Hospital 26 at 6 P. M., September 27, 1918, from Hospital Train No. 63, with a diagnosis of gas, deleterious, absorption of, inhalation and surface contact. Temperature was 98°, pulse 156, respiration 20. The field medical card had been lost in transfer to the train. History obtained stated that he had been gassed three days previously. He was comatose, markedly cyanotic and breathed with great difficulty. The throat was reddened and swollen. The eyes were closed with purulent exudate. There were rales throughout the chest, with bronchial breathing suggesting consolidation. Diagnosis (addition) was made of bronchopneumonia, left lower lobe. September 28, temperature was 99°, pulse 108, respiration 16. Death occurred at 6 A.M.

"*External Appearances.* There is purulent exudate on the conjunctivæ of both eyes, with accumulation at the margins of the upper and lower lids. Small hemorrhages are present on the ocular conjunctivæ laterally to the corneæ. There is a vesicle around the external meatus of the urethra, 2 x 3 cm., and one on the anterior surface of the tibia, 1 cm. in diameter, 7 cm. below the patella. There is another vesicle, 2 cm. in diameter, 1 cm. below the internal malleolus, left.

"*Organs of the Mouth and Neck.* On opening the trachea and larynx there is found marked edema of the mucosa and submucosa. Pin-point hemorrhages, with minute ulcerations, are scattered over the entire epithelium. A purulent exudate covers the surface, very marked over the vocal cords and at the base of the epiglottis. This is firmly adherent to the epithelium. The vocal cords are greatly thickened and the glottis appears almost completely closed through the approximation of the swollen tissues covered by the exudate.

"Lungs: On opening the bronchi they are found to be almost completely filled with tenacious masses of exudate. In the smaller bronchioles these assume a pseudomembranous character. They extend into the still smaller bronchioles as solid plugs, completely closing the lumina. On stripping the exudate the epithelium throughout shows pin-point hemorrhages, with minute ulcerations.

"Left: The lower lobe is large and voluminous. The lower half is feathery. It contains several irregular collapsed areas, reddish brown in color, in contrast to the grayish pink of the rest of the lung. There are firm, irregular masses present, which on section are reddish brown in color and contain grayish areas, 1 to 5 mm. in diameter, from the centers of which pus escapes from the bronchioles. The lower margin of the upper lobe is firm but crepitates slightly. On section, quantities of faintly turbid, blood-tinged fluid escape.

"Right: The right lung has only two lobes. The lower lobe is voluminous, light and feathery. The lower surface of the upper lobe shows numerous air vesicles varying from pin-point size to 2 mm. in diameter, situated along the interlobular septa. On section, quantities of pus escape from the large bronchioles.

"*Anatomical Diagnosis.* Edema of glottis; acute suppurative and ulcerative tracheobronchitis; beginning bronchopneumonia; edema of lungs; acute vesicular and interstitial emphysema; edema and congestion of the brain; congestion of the kidneys; gas burns of the conjunctivæ and skin.

"*Probable Cause of Death.* Acute suppurative tracheobronchitis, with beginning bronchopneumonia and edema of the glottis, following gas inhalation."

*Microscopic Study* (Section 46). The bronchioles are lined with a fibrinopurulent exudate. Some bronchioles are completely plugged. The plugs present a denser infiltration with leukocytes. The mucosa is nearly everywhere absent and the walls are infiltrated with polymorphonuclears. The capillaries are intensely injected. The alveolar walls immediately surrounding the bronchioles also show this injection of the capillaries, but the air vesicles contain relatively very little exudate. A few of the bronchioles are surrounded by a narrow zone of alveoli filled with polymorphonuclear leukocytes and some fibrin. There are small patches of edema in which the albuminous precipitate is very dense, resembling colloid.

Another section of the same lung shows an almost similar picture, but the purulent exudate in the alveoli is somewhat more marked.

CASE II.—Autopsy 25–32. W. B., Corporal, Co. F, 26 Infantry, No. 284685. Aged twenty-three years, white. Died October 14, 1918, at 2.45 A.M. Autopsied October 14, 1918, at 11 A.M. Capt. Moses Barron, M.C., pathologist.

"*Clinical Data.* This is the case of a man who was admitted to Base Hospital No. 25 on October 10, 1918, with extensive skin burns (second degree) over the face, neck, buttocks and thighs, due to mustard-gas contact. Inhalation of the same gas caused a severe inflammatory condition of the respiratory passages. Upon admission the patient was cyanotic, coughed frequently and complained of pains in the chest. Urinalysis showed albumin in the urine and hyalin and granular casts. The patient died at 2.45 A.M. on October 14, 1918.

"Lungs: The lungs and organs of the neck are removed *en masse.* The pyriform sinuses are covered by a thick, fibrinopurulent membrane, which is firm and adherent. The epiglottis is thickened and covered by a similar membrane. The entire larynx, including the vocal cords, presents a thick, shaggy membranous exudate, which, when removed, leaves a congested, necrotic and somewhat ulcerated surface. Portions of the exudate extend for a short distance into the trachea. Farther down into the trachea the mucosa is hemorrhagic, purplish red in color and covered by a thin hemorrhagic and purulent exudate, which becomes more marked in the bronchi and bronchioles where it almost occludes the lumina. On section of the lungs the cut surfaces are mottled, dark brown and red, with yellowish plugs completely filling some of the small bronchioles. On pressure large quantities of frothy fluid flow from the cut surfaces. The organs crepitate only slightly. Some of the bronchioles are surrounded by small grayish yellow, firm areas. The pleura is covered by a thin layer of fibrinopurulent exudate.

"*Anatomical Diagnosis.* Acute fibrinopurulent, hemorrhagic and ulcerative tracheobronchitis, with moderate edema of the glottis; early bronchopneumonia, with edema and congestion of the lungs; acute fibrinopurulent pleuritis; diffuse colloid thyroid; superficial burns of the skin from poisonous gas; mild acute diffuse nephritis.

"*Probable Cause of Death.* Acute fibrinopurulent and hemorrhagic tracheobronchitis, with edema of the lungs and partial edema of the glottis following inhalation of poisonous gas."

*Microscopic Study* (Section 426). The bloodvessels throughout the section are dilated and engorged. The bronchioles are plugged with fibrinopurulent exudate. In two bronchioles these plugs are seen to be attached to the wall at one side, at which point the mucosa is destroyed and the wall is edematous and extensively infiltrated with leukocytes. Remnants of mucous membrane are to be seen on the remainder of the bronchial wall. The capillaries in the wall are markedly engorged with blood. About the bronchioles there is extensive hemorrhage into the alveoli. Patches of hemorrhage alternating with emphysematous areas are scattered throughout the parenchyma. In some portions of the section the alveoli around the bronchioles are filled with a purulent exudate.

CASE III.—Autopsy 26–62. A. R. B., Private, Co. I, 61 Infantry, No. 2253041. Aged twenty-two years, white. Died October 31, 1918, at Base Hospital 26. Autopsied November 1, 1918. Lieut. Oliver C. Melson, M.C., pathologist.

"*Clinical Data.* This case is that of a man who was admitted to Base Hospital 26, October 12, 1918, with a gunshot wound of the right lumbar region. He was suffering from absorption of gas, deleterious, mustard, by inhalation and contact. (Clinical record is not available.) Death occurred October 31, 1918.

"*External Appearances.* . . . There are superficial ulcerations of the ears, eyes, nose and mouth. The skin of the right hand and forearm, both legs, both buttocks and back is pigmented. There are multiple superficial burns of the skin of the right arm, extending from 6 cm. above the elbow to 5 cm. below it on the lateral and flexor surface; on the flexor surface of the right forearm, 4 cm. in diameter; on the left forearm an area, 1 cm. in diameter, 2 cm. above the wrist-joint on the extensor surface, extending 2 cm. upward and inward; and also a smaller, more superficial burn, 5 mm. in diameter. The skin over practically the entire back is involved in superficial burns. . . . The posterior and inner surfaces of both legs, from the upper third of the thigh to 6 cm. below the knee-joint on the left, are involved in superficial burns as well as the anterior surface of the left knee-joint. There are also superficial burns over the external genitalia. There is a debrided wound, partially closed by silkworm sutures and drained by tubes, which extends from the midline (of the back) to the level of the third sacral segment and downward to the left trochanter. The mesial portion of the wound connects with the coccyx, which is fractured. . . ."

"*Organs of the Neck.* The mucous membrane of the trachea and larynx is covered by fibrinopurulent exudate, which is very adherent, appearing as a pseudomembrane. Near the bifurcation of the trachea the mucous membrane is hemorrhagic, with minute ulcerations scattered over the surface.

"Right Lung: The right lung is nodular and crepitant. The pleura is thickened and covered by many firm fibrous adhesions. The cut surface is dark red, moist and studded with irregular round raised areas, reddish in color. From the centers of these areas, yellow, purulent material can be expressed on pressure. Many are soft and necrotic, with a very foul odor.

"Left Lung: The left lung is nodular and crepitant. It is salmon colored and mottled with anthracotic pigment. The lower lobe is covered by shaggy, fibrous adhesions. At the apex of the upper lobe is a firm, grayish, non-aircontaining area, 5 cm. in diameter, which is granular to the feel and on pressure exudes large quantities of yellow purulent material. This area is sharply demarcated from the surrounding tissue. The remainder of the lung corresponds to the right in description, except that the nodules are fewer in number. The bronchi contain a considerable quantity of frothy bloody fluid. The mucous membrane is markedly injected.

*Anatomical Diagnosis.* Advanced bilateral bronchopneumonia, with necrosis and abscess formation; acute hemorrhagic, suppurative and ulcerative tracheobronchitis (due to gas inhalation); multiple superficial skin burns; gunshot wound of the left buttock, with compound comminuted fracture of the coccyx; chronic adhesive pleuritis.

"*Probable Cause of Death.* Acute suppurative and ulcerative tracheobronchitis and bronchopneumonia."

CASE IV.—Autopsy 56–68. A. R. M., Private, Co. H., 312 Infantry, No. 3754421. Aged twenty-one years, white. Died January 3, 1919, at 3.45 P.M., at Base Hospital 56. Autopsied January 4, 1919, at 9.30 A.M. First Lieut. George W. Covey, M.C., pathologist.

"*Clinical Data.* This is the case of a man who was admitted to Base Hospital 26 on October 24, 1918, with the history of having been exposed to mustard gas on October 18. There were no skin burns, but the eyes were much inflamed. Examination showed the patient to be quite cyanotic, with marked conjunctivitis, accompanied by purulent discharge and ciliary injection. The pharynx was much injected; pulse good; heart negative; lungs showed many whistling rales throughout, with crackling rales in both bases most marked in the left posteriorly. No definite consolidation. Temperature 102°. October 27, smear negative for B. diphtheria. Dulness and suppression of breath sounds in areas in the right axilla and the right base posteriorly. November 25 he was much improved; still much semipurulent expectoration and cough; chest full of moist and sonorous rales. Heart rapid. Persistent afternoon temperature of 100°. November 30 the throat culture was positive for diphtheria and 10,000 units of diphtheria antitoxin given. He was transferred to Base Hospital 56 on December 1, with very marked membrane on both tonsils. Marked hoarseness. Given 30,000 units of diphtheria antitoxin at 3 P.M. December 3 was given 20,000 units of antitoxin at 9.10 P.M. December 4 the examination of the chest showed marked bronchial breathing all over the lungs, with many rales of all characters. Diagnosis: acute diffuse bronchopneumonia, bilateral, secondary to absorption of mustard gas; diphtheria, pharyngeal and tonsillar. He was given a total of 120,000 units of antitoxin. December 10 the patient had an attack of dyspnea lasting a few minutes. Examination showed respiration of asthmatic type, at the bases a few mucous rales, with areas of consolidation. There was a partial paralysis of the palate. December 17 his condition was about the same; sputum negative for tubercles. December 23 the patient had consolidation of right base of bronchopneumonic type. Quite dyspneic. January 3, 1919, his condition was worse; unable to take nourishment; bubbling rales throughout lungs. Died at 4 P.M., January 3.

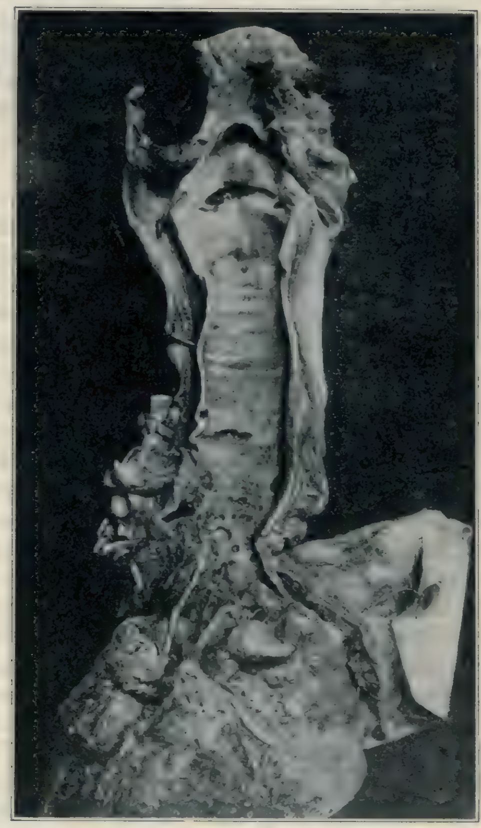

FIG. 1.—Larynx and trachea two months and ten days after gassing (Aut. 55–68, Case IV in text). Note extensive ulcerations, most marked at the bifurcation. The large transversely placed ulcer in the lower part of the trachea perforated through a cartilaginous ring.

"Pleural Cavities: The left pleural cavity has a strong band of adhesions at the apex. There is no excess of fluid. The right also has a strong band of adhesions at the apex and no increase in fluid.

"Lungs and Organs of Neck: The organs of the neck and the lungs are removed *en masse*. The esophagus is normal. The tonsils, pharynx and upper portion of the larynx are normal. Just below the posterior extremity of each vocal cord there is an opening, about 5 mm. in diameter, leading into a cavity which extends into the laryngeal wall for a distance of about 1 cm. Pus can be expressed from these cavities; otherwise the larynx and upper 5 cm. of the trachea are normal. Beginning about 5 cm. below the larynx and continuing downward throughout the trachea and bronchi the mucosa is dark greenish in color, hemorrhagic and covered with pus.

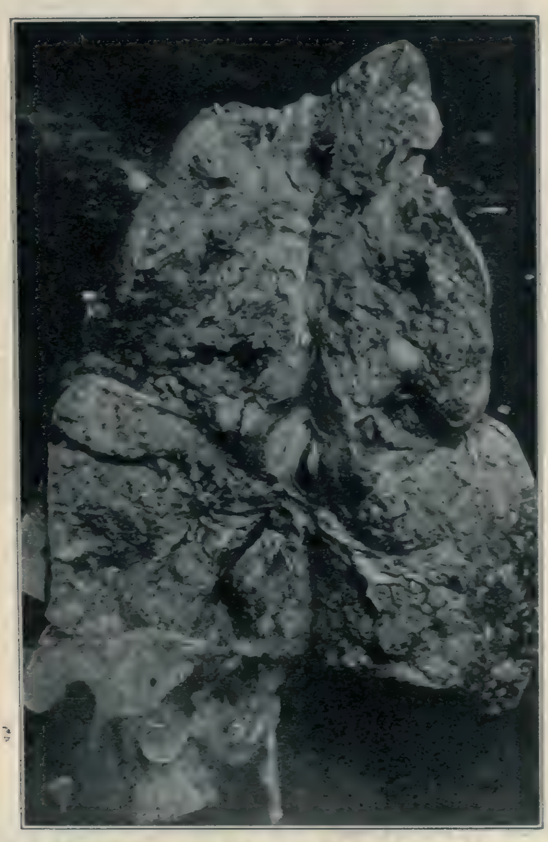

FIG. 2.—Lung from same case as Fig. 1. (The trachea and larynx are lying back of the lung, with the epiglottis and base of tongue showing just beneath the base of lung). Note the numerous areas of consolidation scattered throughout the cut surfaces. In the centers of many of these the emptied lumina of the bronchioles may be seen. Note the ragged areas of necrosis and abscess cavities.

About 5 cm. above the bifurcation there is an ulcer with undermined edges, extending through the mucosa and through a cartilaginous ring to the outer coat of the trachea. It measures 1 cm. in diameter. From this point downward there are similar ulcerations becoming more and more numerous (Fig. 1).

"The left lung is voluminous, heavier than normal. There is a scar at the apex. Numerous hemorrhages are present beneath the pleura. There is marked vesicular emphysema. The cut surface of the upper lobe reveals a group of calcified nodules beneath the scar. The color is normal. Large quantities of pus are expressed from the bronchi and plugs of thick white exudate are expressed from the smaller bronchi and bronchioles. The cut surface of the lower lobe is dark red in color and practically every bronchus contains a plug of exudate which projects above the surface. Frothy bloody fluid exudes from the parenchyma.

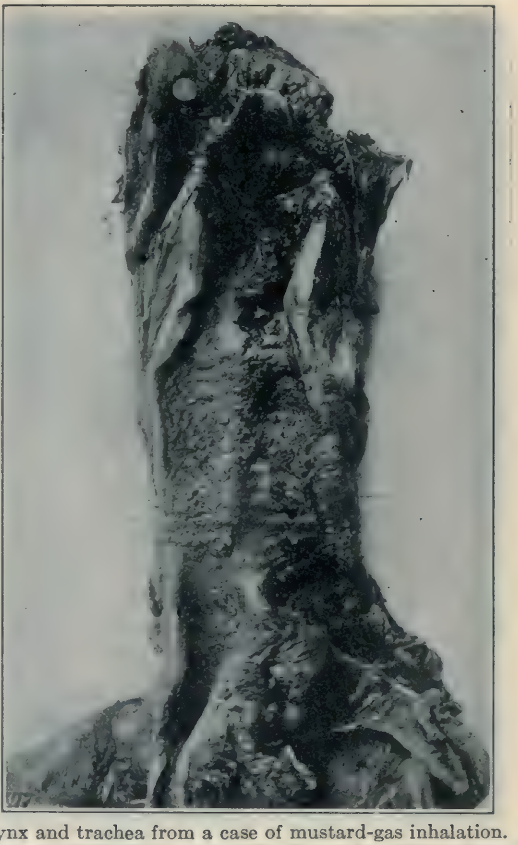

FIG. 3.—Larynx and trachea from a case of mustard-gas inhalation. Note extensive edema of aryepiglottic folds especially the right; thick, shaggy membrane covering the larynx and vocal cords; membrane in patches on the wall of the trachea and bronchi; small ulceration of the wall near the bifurcation.

"The right lung is heavy, large, firm, with marked vesicular emphysema and numerous hemorrhages beneath the pleura. The pleura is covered by a fibrinopurulent exudate. The cut surfaces of the upper and lower lobes are similar and as follows: Several large, ragged, irregular abscess cavities filled with thin grayish, granular pus, are scattered through the central portions of these lobes from the apex to the base (Fig. 2). They vary from a few millimeters to two or more centimeters in diameter. The central portion of both lobes is an airless, gray, granular mass—a central pneumonia. Toward the pleura the pneumonic areas become more discrete and are seen as gray, raised, granular areas varying from 3 or 4 mm. to 1 cm. or more in diameter, quite definitely circumscribed. In the center of each is a bronchiole from which a plug of exudate can be expressed. Occasionally the center of a larger discrete area is seen to be necrotic, with a small abscess cavity (Figs. 1 and 2). The parenchyma intervening between the pneu-

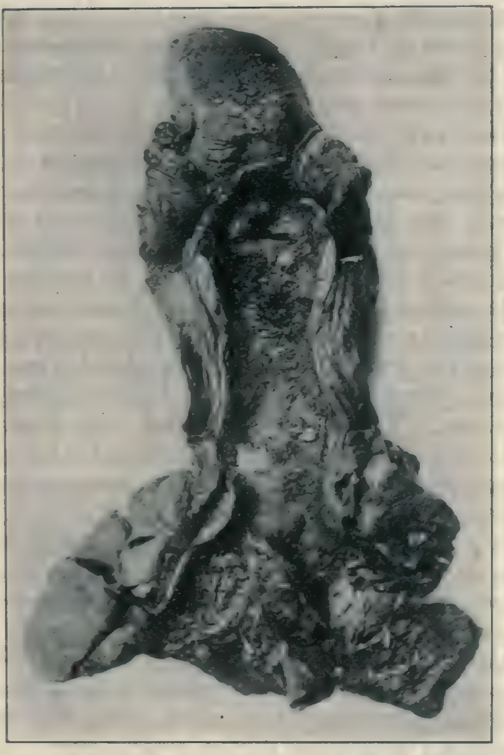

FIG. 4.—Larynx and trachea from case of mustard-gas inhalation. Note thick membrane covering epiglottis, obscuring the vocal cords and extending in patches down the trachea; thick membrane on the walls of the primary bronchi; small ulcerations of tracheal wall near the bifurcation.

monic areas is dark red in color and emphysematous, with areas of hemorrhage. Near the hilus are scattered areas showing interlacing, firm yellowish streaks suggesting organization.

"*Anatomical Diagnosis.* Marked gangrenous and ulcerative tracheobronchitis; marked fibrinopurulent bronchitis of smaller bronchi; massive bronchopneumonia, right, with extensive necrosis and abscess formation; healed pulmonary tuberculosis; right-sided fibrinopurulent pleuritis; chronic adhesive pleuritis; marked vesicular emphysema.

"*Probable Cause of Death.* Gangrenous and ulcerative tracheobronchitis; bronchopneumonia, with abscess formation—following inhalation of poisonous gases."

*Microscopic Study* (Slide 449). The striking feature of the section is a small bronchus dilated and filled with pus cells. In the center of this mass is a group of vegetable cells and spirals. The wall of the bronchus is only faintly visible, being infiltrated with polymorphonuclear leukocytes. The mucosa is entirely absent. The capillaries are intensely injected and dilated. In two places the wall is completely necrosed, and this necrosis extends for some distance into the parenchyma of the lung. About the bronchus and throughout the section there is thickening of the alveolar walls, which are infiltrated with polymorphonuclears and show a large increase of connective tissue. The alveoli are filled with fibrin and heavy precipitated serum through which are scattered a few cells. Fibroblasts are seen penetrating the exudate in numerous instances, and some alveoli are completely fibrosed.

DISCUSSION. Case I died the day after admission, and, allowing one day for transportation from the field, about five days after gassing. Here are seen, as the outstanding features, edema, congestion, hemorrhages and very small ulcerations, with thin fibrinopurulent exudate in the upper respiratory tract. In the lower air passages one finds much the same picture with a more tenacious exudate which almost completely plugs many bronchioles. The parenchyma shows congestion, edema, emphysema, areas of atelectasis and very small bronchopneumonic areas.

Case II died about six days after gassing. The exudate in this case is much more abundant than in Case I, and is shaggy and membranous. The ulcers in the trachea are a little more prominent. There is already a pleurisy present.

Case III died twenty days after gassing. The air passages show a marked pseudomembrane, and, upon its removal, a hemorrhagic and ulcerated surface. The lungs showed an advanced bronchopneumonia, with beginning necrosis and abscess formation in the center of some of the pneumonic areas.

Case IV died two months and ten days after being gassed. The air passages, trachea and bronchi show no membrane, but instead show marked necrosis of the mucosa, with large, deep ulcerations extending nearly through the tracheal wall. The lungs show extensive necrosis and abscess formation, accompanying a massive bronchopneumonia. The attempt at healing is shown by the organization of the exudate. Many alveolar walls are thickened and some alveoli are completely fibrosed. Strange to say, much albuminous deposit and fibrin are still in evidence in the air cells. Unfortunately, this case was complicated by clinical diphtheria, but most probably this complication did not materially change the lesions found at autopsy. Only one finding brings up a question of etiology: the evidences of the aspiration pneumonia as revealed by food particles lying imbedded in the purulent exudate within the small radicles of the bronchi. Was the aspiration of the material due to the paralysis following the diphtheria or to the deep ulcers in the trachea opposite the esophagus? The abscess formation might also have been augmented by the aspiration of the foreign material.

To recapitulate: Stage one, as represented by the first two cases, is characterized by mild edema, hemorrhage, beginning ulceration, early fibrinous deposit, emphysema and very early bronchopneumonia. Stage two (Case III) by a marked fibrinopurulent pseudomembrane, more extensive ulceration, emphysema, advanced bronchopneumonia and beginning necrosis. Stage three (Case IV) by ulceration, necrosis and abscess formation, with massive bronchopneumonia. The attempts at healing are revealed by the organization and fibrosis.

It is interesting to speculate on the outcome of a case similar to the fourth should such a case go on to recovery or if the lesions in the trachea should continue to progress. The tracheal ulcerations might easily perforate the wall, resulting in mediastinal abscess. Those in the bronchi near the bifurcation might ulcerate through and through and perforate the aorta. When such ulcers heal, contractures are sure to obtain. These contractures may result in traction diverticulæ of the esophagus. In the parenchyma, organization would occur and result in disseminated patches of fibrosis or even in extensive fibrosis of the lungs resembling healed tuberculous lesions. Some of the bronchioles might be transformed into fibrous cords through the organization of the exudate.

In milder involvement the walls only might go on to a fibrosis of a greater or lesser degree, which would result clinically in a chronic bronchitis. Upon the extent of the injury to the bronchial tree will depend the intensity of the clinical symptoms of convalescent and recovered cases, as manifested by the chronic bronchitis and emphysema. From what has been said above, many cases will undoubtedly present grave clinical difficulties in the differential diagnosis from pulmonary tuberculosis.

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# DIPHTHERIA AT A HOSPITAL CENTER

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Havard[1] states that diphtheria is "a disease of minor importance in the Army." He quotes statistics to support this view. Keefer[2] and others, however, find that an epidemic of this disease is at times very difficult to control. It has been the experience of the writers at the Hospital Center, Allerey, Saone et Loire, France, that it requires at times considerable effort, ingenuity and cooperation between the laboratory, ward surgeons, nursing corps and patients, to bring about the desired results. In one-story wooden barracks and tents, covering less than one square mile of space, there were over 17,000 patients and 3,000 personnel at the time that the disease was at its maximum. It is evident that the crowding was very great, and this condition always hampers the control of a contagious disease.

## Epidemiology

The first case of diphtheria on record at this Center occurred in Ward V, Base Hospital 26, Sept. 24, 1918. There were two more on Oct. 1, one on the 5th, one on the 8th, three on the 9th, and so on until in the period of Nov. 10 to Dec. 8, from one to seven cases were reported daily. The incidence of clinical cases and carriers at each base hospital is shown in chart 1. Chart 2 is a composite of all the cases in the entire Center. The three hospitals (26, 25 and 49) in which the first cases appeared, were the ones in which more than two-thirds of the entire number of cases developed. It should be borne in mind that since these three hospitals were the first to arrive at this Center, they received the largest number of patients. In these hospitals several nurses and ward men who had been known to be carriers in the States were again identified as such here, but only after the infection had been under way for some time and the culturing had become general. Such chronic carriers might have been factors in introducing the disease at this Center, but it is also possible that they were reinfected here. It may perhaps be unjust that a person once found to be a diphtheria carrier should thereafter always be blamed for any diphtheria developing in the vicinity, but the possibility of course cannot be denied. One of the supposed carriers had only a true Hoffman's bacillus morphologically and culturally, since it did not ferment any of the sugars.

Very early in the epidemic carriers were found with striking frequency among the gassed cases. A number of these cases, diagnosed as diphtheria because of positive throat cultures, came to postmortem examinations and

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[1] Military Hygiene, p. 91.

[2] Keefer, F. R., Friedberg, S. A., and Aronson, J. D.: Jour. Am. Med. Assn., 1918, 71, p. 1206.

showed either no membrane at all or only a small and clinically insignificant one. Yet from the larynx of these cases practically pure cultures of typical B. diphtheriae were obtained. The relative frequency with which such cases were encountered suggested a second possible mode for the introduction of the organism at this Center.

The first ward to be under quarantine for any length of time, due to the constant discovery of new carriers, was the pneumonia ward at base hospital 26, where there were a large number of gassed cases. The same was true in base hospital 56. In base hospital 25 the first cases of diphtheria appeared in the gassed wards. At one time there were 75 known diphtheria carriers (chart 5, B) of whom 33⅓% gave histories of gassing; whereas, of the 35 clinical cases only 5.7% gave such a history. As a possible explanation of the marked preponderance of carriers over clinical cases, it is suggested that the inflammatory changes in the respiratory mucous membrane incident to gassing produces an environment favorable for bacterial growth. The pneumococcus and particularly the streptococcus are invasive organisms frequently causing pneumonia in such cases, but the diphtheria bacillus is relatively noninvasive. Consequently, while the antitoxin producing power of the patient remains unimpaired, diphtheria bacilli may be present in large numbers without causing the disease. Such cases are carriers. It was impossible to go on with this phase of investigation since with the cessation of hostilities no more gassed cases arrived.

Crowding, which means droplet infection, was probably the chief factor in the spread of diphtheria at this Center. During October and early November all wards were greatly overcrowded. Designed for 50 beds, they held 70. To many of the wards were attached one or more tents of 50 beds each, with only just space enough to crowd between every second bed. In the wards, two patients had to share the same table. There were barely sheets enough to cubicle the acute respiratory wards.

When the pneumonia ward of base hospital 26 was first quarantined it was very striking how, over a period of some 2 weeks, carriers continued to appear in two of the tents connected with the ward, whereas the third tent remained uninfected, though cultured as often as the others. This result can be explained only by strict isolation of the uninfected tent from those which were infected. or, due to the crowding, had the organism once been introduced, it would undoubtedly have spread from one end of the tent to the other in a comparatively short time, just as it had in the other two.

The indirect spread of infection by hands and fomites as opposed to direct spread by droplet infection has been known for a long time.[3] Fumigation in all its aspects is intended to combat this. But of late the idea that a contagious patient in bed may infect the outmost corners of his room has been replaced by that of direct droplet infection incident to coughing, talking, sneezing, etc., so that only objects in immediate contact with a patient are considered dangerous. Supreme emphasis is therefore placed on the masking of all in attendance. The pendulum has swung a little too far, as our investigation would suggest, from indirect transmission through hands and fomites to direct transmission by means of mouth to mouth droplet infection. This phase of the investigation was suggested by Lieut.-Col. Witherspoon, and it seemed that in the typical diphtheria bacillus we had an unusual opportunity for study, since this organism, we thought, could be recognized in culture by its morphology alone. Also we had here a considerable number of clinical cases and contacts under far better control than is possible in civil life.

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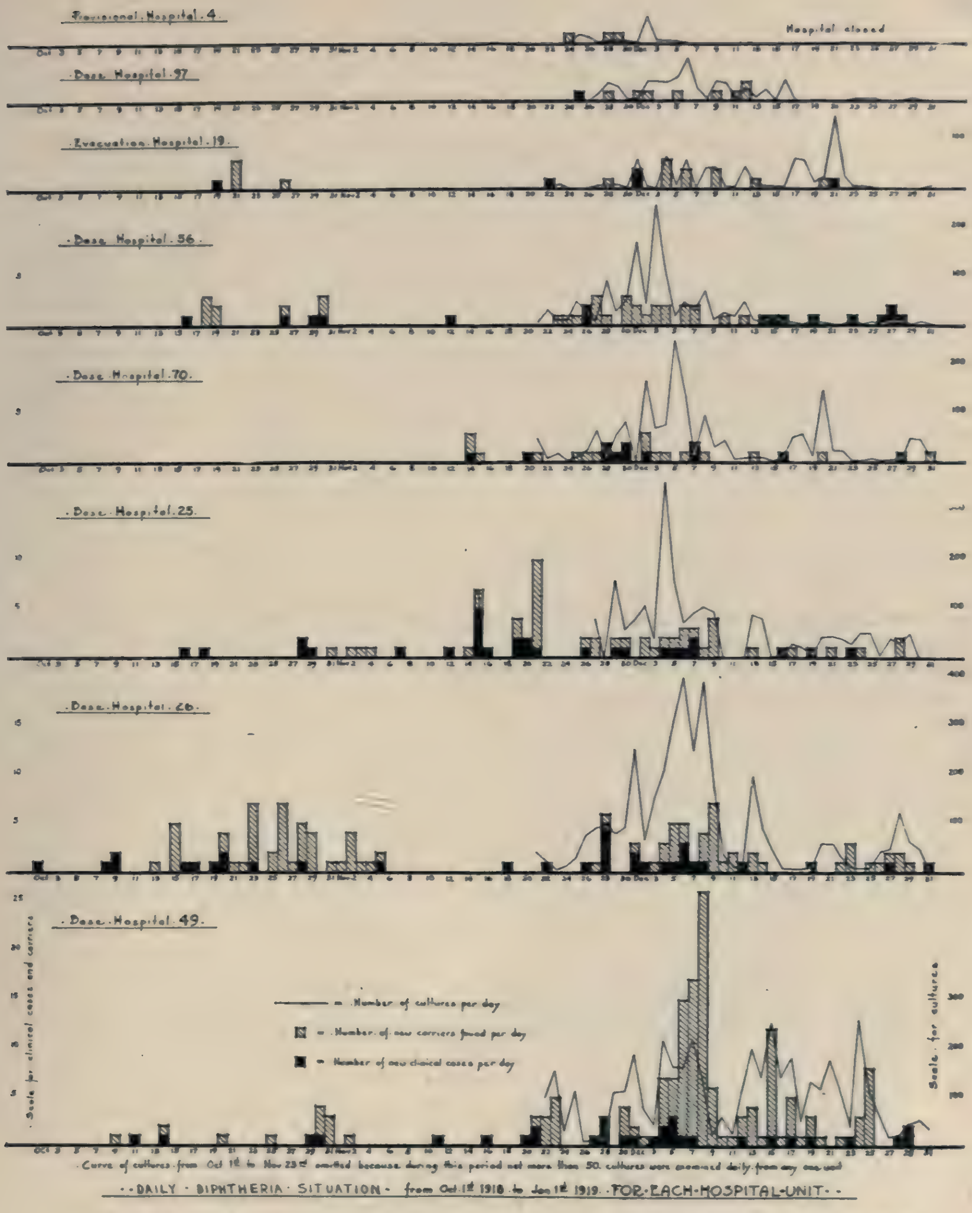
[3] Park, W. H., and Williams, A. W.: Pathogenic Micro-organisms, 1917, p. 297.



Curve of cultures from Oct 1st to Nov 23rd omitted because during this period not more than 50 cultures were examined daily from any one unit

DAILY DIPHTHERIA SITUATION from Oct. 1st 1918 to Jan 1st 1919. FOR EACH HOSPITAL UNIT

Chart 1.—It should be noted that base hospitals 49, 25 and 26 furnished most of the clinical cases and carriers. Up to Nov. 6, three to four carriers were found at base hospitals 49 and 26 for each clinical case, whereas, compared with the number of clinical cases, base hospital 26 showed few carriers. This may in part be accounted for by the less general culturing at this hospital at this time. The clinical case at base hospital 26 recorded on Oct. 1 was the first case at this Center and should have been recorded on Sept. 24, had the chart permitted. The first carrier at provisional hospital 4 was sent to base hospital 56 as a clinical case, but it was decided to be merely a follicular tonsillitis with a positive culture. On Dec. 6, 7 and 8 the marked increase in carriers at base hospital 49 was due to the great prevalence of the atypical forms. The same were found Dec. 8 at base hospital 26, when the carelessness was shown in taking cultures. These were never reported positive, since the ward was quarantined and all throats cultured. The same procedure was adopted at base hospital 49, as is shown by the marked drop in the curve of cultures Dec. 9. The most recent cases recorded at base hospital 56 were in patients held in the cubicle and mask observation ward, which may account for the absence of new carriers. It should be noted that in all instances the sum of chart 1 will not equal chart 2. This is because some 13 of the first clinical cases could not be traced back to the base hospital where they originated, and some few cases were sent in from the convalescent camp, which is not shown on chart 1.

The diphtheria wards, both clinical and carrier, were chosen for this investigation, using wards in which no cases of diphtheria had developed as controls. In all, 522 cultures were made. The following procedure was adopted: a sterile swab moistened in sterile broth was wiped over the palm, particular attention being paid to the creases. It was felt that owing to heat and moisture the creases would be most likely to retain the organisms which might be deposited there. The swab was inoculated on Loeffler's blood serum and incubated from 16-18 hours. Working on the principle suggested by Williams,[4] some 90 cultures were enriched after 8 hours' incubation by inoculating fresh tubes with a loop passed over the surface of the original one. Both series of tubes were examined from 10-12 hours later. The enriched series did not show a constant increase in the number of positives and the procedure was discontinued. A few cultures were first taken from the fingers also, but this was soon abandoned because no additional information was obtained. It was felt that contamination of the fingers could be reasonably assumed once the palm was proved positive.

All the attendants and nearly all the patients were masked in the clinical and carrier diphtheria wards. A few of the bed patients who were cubicled were not masked Occasionally the patients were asked to cough before the cultures were taken. Patients without masks and often patients with masks would unconsciously place their hands on their mouths when coughing. At times they were explicitly told to cough into their hands. This was done routinely toward the latter part of our investigation.

For purposes of comparison patients were divided into two groups: Those in "infected" wards, and those in "uninfected" wards. We use the term "infected" wards to mean wards in which clinical cases or carriers were isolated. Base hospital 56 furnished the "infected" wards because all clinical and carrier cases from the Center were sent there. The term "uninfected" refers to wards in which no clinical case or carrier had been identified at any time; such wards were selected from hospitals in which there had been the least amount of diphtheria. These groups were again divided into "masked" and "unmasked." Unmasked patients were rather hard to find in the diphtheria wards, with the exception of the cubicled bed patients. In the "uninfected" wards chosen, all were unmasked. Fomites were similarly subdivided into "masked" and "unmasked." Only articles used or handled by masked patients were considered "masked" fomites. Such articles included door-knobs, handles on latrine pail covers, pokers, crutches, and personal possessions such as mess-kits, mirrors, rosaries, magazines, foot-rails of beds, etc. The personal possessions of unmasked bed patients were considered "unmasked" fomites. This distinction is entirely arbitrary, since cigaret papers, spoons, mirrors, combs, etc., are used when the mask is off and the patient who is masked at the time of culture may have had his mask off or hanging from one ear only 5 minutes previously.

The typical diphtheria bacillus when present in these cultures was found only after considerable search. They were usually in clumps of three or more, showing polar-staining and parallel or stellate arrangement. The majority were of the smaller forms corresponding to Wesbrook's "C" or "D". The atypical diphtheria bacillus or diphtheroid was similar in morphology to that found in the throat, in the mouth, in the anterior nares, and in several of the wounds. It resembles the diphtheroid described[5] as the normal inhabitant of the skin.

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[4] Ibid., p. 306.

[5] Eberson: Jour. Infect. Dis., 1918, 23, p. 1.

The number of the organisms showing metachromatic polar granules was very surprising. Some of these were often indistinguishable from the large polar-staining diphtheria, though the frequency of a large metachromatic granule at the center made one at once suspicious. Also the majority did not show the characteristic tapering toward the center nor the usual slight curve. When the grouping of these organisms in the smears was considered, the absence of all palisade arrangement was striking, the bacilli often appearing in chains of two, three or even more. Spore-bearing bacilli were found in the same smears. These two forms were occasionally the only organisms present. It is, of course, unreasonable to expect to recover an almost pure culture of diphtheria bacilli in cultures from the foot of a bed or from the hand of a patient, even when the patient is known to harbor this organism in his throat. It was felt that the spore-bearing and the polar-staining bacilli were probably only different forms of the same organism—a saprophyte corresponding to Bizzozero's Leptothrix epidermis and not a diphtheroid at all. To prove this, isolation was attempted. Isolation through plating failed because of the extremely spreading character of the growth. Isolation of the spore-bearer through heating, however, was successful. Suspensions of the

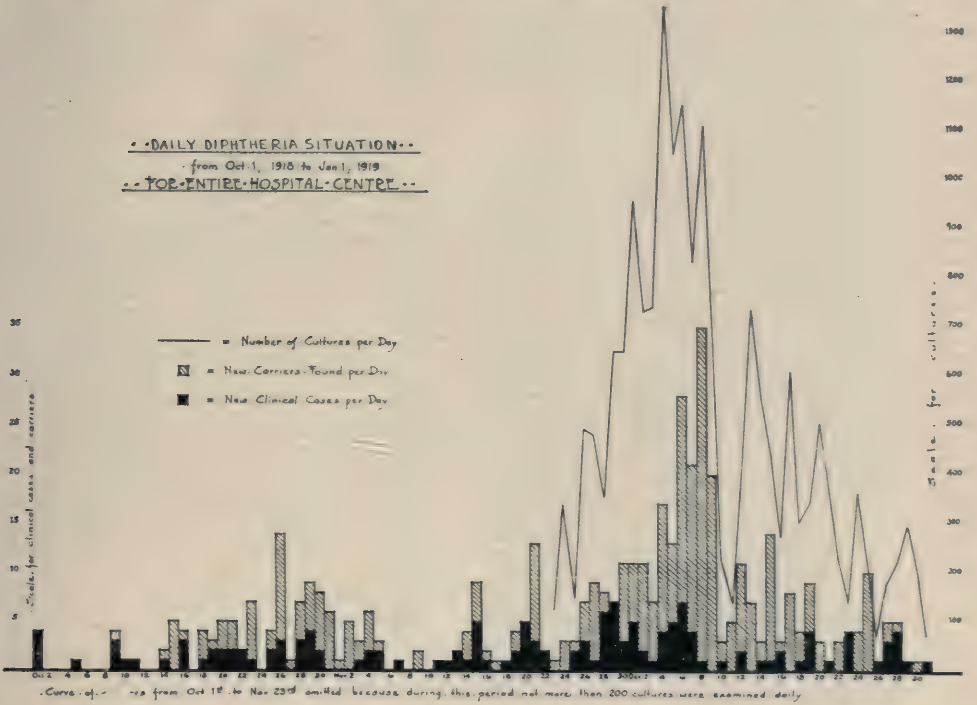

Chart 2.—Note that in general the curve of new carriers found follows that of the number of cultures examined. The marked exception is on Dec. 8, when the atypical form was being found in great numbers at base hospital 49. In Oct. and early Nov. the culturing was much less general, only those immediately exposed being cultured. Hence, there was at that time a much higher proportion of positives.

culture in salt solution were heated for half an hour at 70 C. and then planted out on Loeffler's blood serum. As controls were used cultures of typical diphtheria from a clinical case which killed a guinea-pig in 36 hours; a culture of typical diphtheria recovered from a larynx at necropsy; a culture from a wound showing type "D 2" organisms. All the controls showed no growth in the subcultures at the end of 72 hours. The subculture of the spore-bearer, however, showed an abundant growth at the end of 24 hours with both the polar-staining and the spore-bearing forms present. These were identical with the original culture. Cultures from the foot of the bed, from a hand, and from a rosary, all showing these two forms, had the same forms in the subcultures after heating. This gave us convincing proof that this polar-staining form was not of the diphtheria group at all, though in occasional instances it would have been extremely difficult to exclude it without the examination following heating.

Chart 3 shows results of the 522 cultures from hands and fomites; 16.1% of the "unmasked" hands in "infected" wards showed typical diphtheria, while only 6.4% of the "masked" hands showed it, thus indicating the value of masking in preventing droplet infection of the hands. Of the cultures from unexposed wards, 5.4% showed a diphtheroid and none typical diphtheria bacilli. The incidence of this diphtheroid in cultures from exposed wards was much higher, 10.3 %; in these wards patients were known to harbor a morphologically similar organism. However, since these organisms or similar organisms are normal inhabitants of the skin, the results of the investigation as far as this organism is concerned, may be somewhat misleading, because its presence may be accounted for otherwise than by droplet infection from the throat. But when a typical diphtheria bacillus is found on the hand and at the same time is known to be an inhabitant of the patient's throat, it is reasonable to assume a direct transmission from throat to hand by coughing or sneezing, and thence to fomites by the contaminated hand. As noted above, this study would emphasize the value of masks in preventing droplet infection of hands and probably of fomites, but whether direct droplet infection from mouth to mouth or indirect droplet infection by way of the hands and fomites is the principal mode of spread must still be little more than a matter of individual opinion. However, this does emphasize what has long been known—the importance of the indirect method of spread of diphtheria. Greater attention must be paid to this manner of disseminating contagious diseases.

A third factor in spread, once the organism had been introduced, was late diagnosis. Because of the considerable number of gassed patients in the hospitals, recognition of laryngeal diphtheria was at times extremely difficult. This was due to the great similarity between the two diseases, not only in the mechanical obstructive symptoms which developed but also in the appearance of the membranous exudates. The severely gassed cases often developed fibrino-purulent membranes which covered the lining of the larynx and trachea, extending from the epiglottis down into the bronchi and bronchioles. The aryepiglottic folds and tissues about the ventricles and vocal cords were at times markedly edematous, producing voice changes, hoarseness and mechanical obstruction to breathing. This membranous exudate rarely extended upward into the pharynx and never over the tonsils. But nearly all of the serious cases of diphtheria had severe laryngeal involvement, often without any pharyngeal manifestations, so that even at necropsies it necessitated close scrutiny to differentiate between the two types of lesions. Besides, diphtheria was sometimes superimposed on the laryngitis following gassing. It is there-

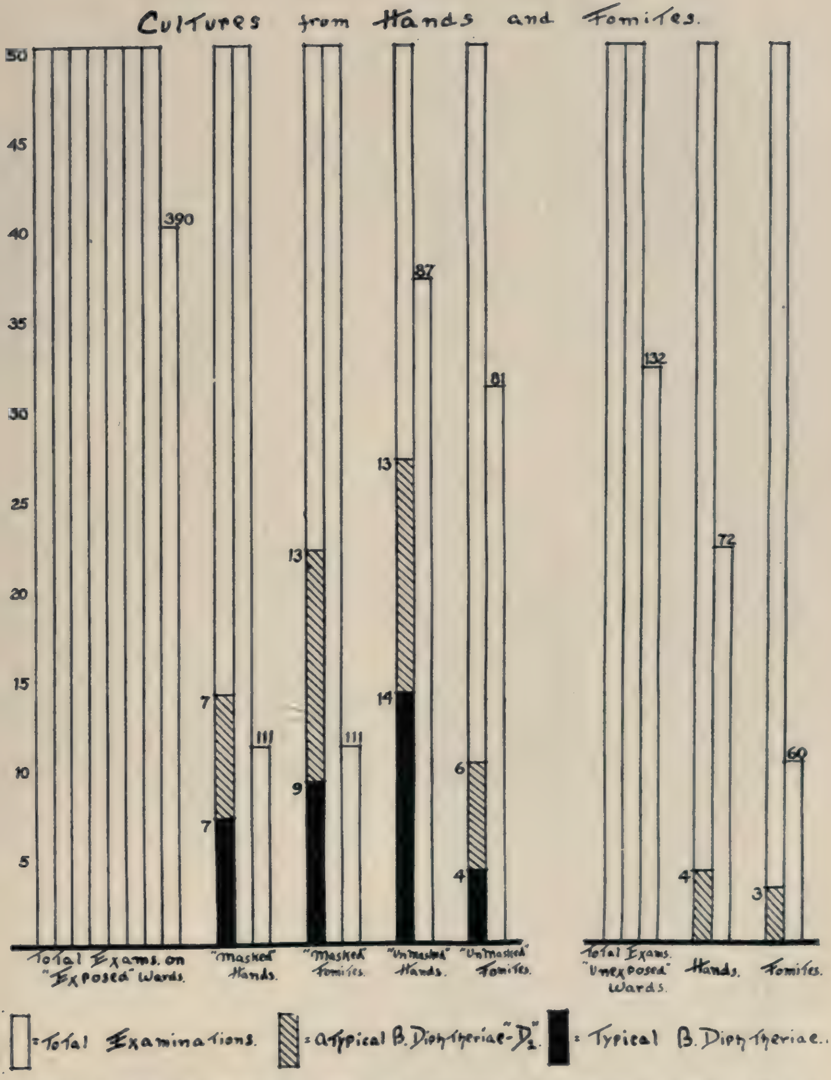

Chart 3.—"Exposed" Wards: Typical B. diphtheriae on "masked" hands, 6.3%; on "masked" fomites, 8.1%; Typical B. diphtheriae on "unmasked" hands, 16.1%; on "unmasked" fomites, 4.9%; Atypical B. diphtheriae on "masked" hands, 6.3%; on "masked" fomites, 11.7%; Atypical B. diphtheriae on "unmasked" hands, 14.9%; on "unmasked" fomites, 7.4%. "Unexposed" Wards: Atypical B. diphtheriae on "unmasked" hands, 5.9%; on "unmasked" fomites, 5.0%. "Exposed" wards were those in which the clinical cases of diphtheria or carriers were treated. "Unexposed" wards were those in which no cases of diphtheria or carriers had been found at any time. The term "masked" means that the patient whose hands or fomites were cultured wore a mask, while "unmasked" means conversely that he wore no mask. It should be noted that typical B. diphtheriae was found nearly three times as often on the hands of those not wearing masks as on those wearing them. Nearly the same difference was found with the atypical form. With the fomites the "masked" were more often infected. This may be explained in part by the fact that many of the fomites considered "masked" were used only when the patients were unmasked. None of the control cultures on "unexposed" wards showed typical B. diphtheriae.

fore easily seen that there were great difficulties in differential diagnosis. As a result an order was sent out that on admission to the hospital all gassed cases must be cultured for diphtheria.

Three cases came to necropsy in which laryngeal diphtheria was found to be the cause of death but which were not diagnosed clinically. Two more patients died of laryngeal diphtheria in which the diagnosis was made only 24 hours before death. Such cases obviously are a great menace in congested wards. Another case of delayed diagnosis was that of an enlisted man who came to the laboratory for examination accompanied by an attendant from one of the medical huts. He had been sick for 3 days, sitting around the stove in his barracks. There was marked respiratory stridor, some aphonia, and considerable swelling of the neck which had been painted with iodin. On inspection he showed a perfectly characteristic membrane over both tonsils extending up onto the soft palate. Direct smears from the exudate showed the diphtheria bacillus. Such experiences of delayed diagnoses help explain the spread of diphtheria in spite of all other precautions.

## BACTERIOLOGY

During our study of diphtheria at this Center, beside the typical polar-staining bacillus of Klebs and Loeffler, we found a small diphtheroid in large numbers from cultures of the nose and throat. Our attention was called to this organism early, when a ward at base hospital 49 was cultured. Two cases showed this bacillus in practically pure culture with no typical polar staining, and the ward was pronounced negative. Three days later a new clinical case appeared in this ward. This led us to report the first culture, in which this diphtheroid appeared as "Diagnosis reserved," and to request a new culture. If the organism persisted, the report "Positive atypical" was made. This arbitrary standard was to be followed until we had time to obtain results as to its virulence and its frequency in clinical cases.

This diphtheroid is a lancet- or wedge-shaped diplobacillus slightly resembling pneumococcus but somewhat longer in proportion to its width. It shows the palisade arrangement characteristic of the diphtheria group, and tinctorially and morphologically corresponds to "$D_2$" in Wesbrook's classification. It is gram-positive, nonmotile, shows no spores and no capsule. As a routine in the laboratory we used Barron's polychrome toluidin-blue[6] diluted 1:5, which stains it uniformly except for the midfissure. There are neither barred nor polar forms. We have found that this form may go over into the typical staining organism, may be mixed with it, or may remain entirely true to type. For example, a throat culture has not infrequently been brought to the laboratory in the morning, which after some 8 hours' incubation showed only the wedge forms; whereas, on the following morning only typical polar forms were present. Again, at base hospital 26 one of the gassed cases, followed by pneumonia, was negative when examined in a routine culture of the quarantined ward. Two days later the patient showed a few of the atypical forms, and these were practically in pure culture the next day. A culture on the

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[6] Jour. Lab. and Clin. Med., 1918, 3, p. 432.

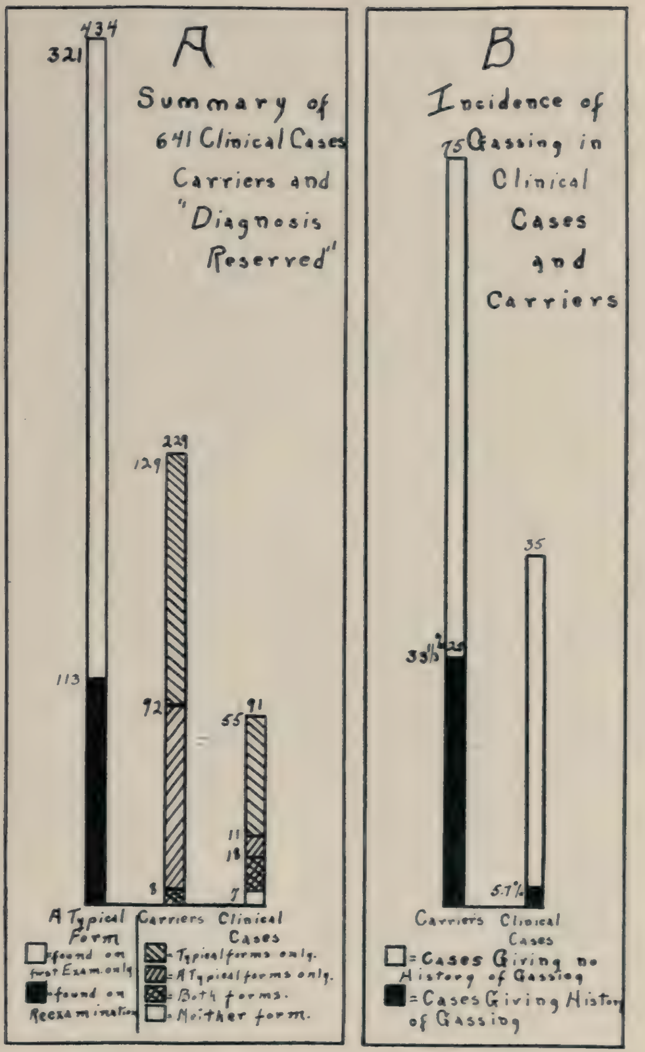

**Chart 4.—A: "Diagnosis reserved" was the report given on cultures when the atypical** form was first found. A second culture was requested and if the organism persisted the 2nd day the report "Positive atypical" was given. The first column shows that in about three out of every four cases the atypical form did not persist, that is, it was but a transient inhabitant of the throat. Note that the atypical form only was found in 56.1% of the carriers and 60.4% of the clinical cases; the atypical form only appeared in 40.1% of the carriers, and 12% of the clinical cases; both forms were found at different times in 3.8% of the carriers and 19.7% of the clinical cases. The 7.9% of the clinical cases in which no organism of the diphtheria group was found is accounted for as follows: At first all the individual cultures, among which were most of the clinical cases, were examined at the unit laboratories. If found positive the patient was sent to the contagious hospital, where, owing to the stress of work, no other culture often was taken until the patient was convalescent and ready for discharge. In such cases he would not appear on our records until late and then occasionally as having only negative cultures. B: This chart is made from patients found in the carrier and clinical wards at a given time, and not from selected cases. Unfortunately the percentage of gassed cases of the total number of patients in the hospital was unobtainable. But it is striking that although the carriers and clinical cases came from the whole Center at large, 33⅓% of the carriers gave a history of gassing, while only **5% of the clinical cases gave such a history.**

following day showed typical B. diphtheriae. At necropsy smears from the larynx showed the characteristic organisms, though there was no evidence of any membrane. Some of the throat cultures showing only wedge forms in original culture showed a mixture of wedge, polar, barred and club forms after isolation in pure culture (chart 4).

The cultural characteristics of this form are not unlike those of typical diphtheria. On Loeffler's blood serum, at the end of from 12-16 hours, it shows small, discrete, glistening, buff-gray colonies, slightly raised. Toward the butt of the tube, where the medium is moist, they become confluent. On broth there is moderate clouding, at times flocculation, but no pellicle formation. The relation of this organism to the typical diphtheria bacillus is one of great interest. Table 1 shows the morphology and sugar reactions on 14 cultures isolated early. Six are from clinical cases, of which, in the original cultures, 4 showed the typical organisms, and 2 the wedge form only. When isolated in pure culture all showed the typical form. The other 8 cultures were from carriers, 2 of which showed typical diphtheria, and the 6 others showed a preponderance of the wedge form.

*Technic of Isolation.*—A small loop of material from the original throat culture was streaked out on one-half of a 1% dextrose-agar plate, to which one-half of 1 c c of human serum had been added. From this a suspicious colony, usually pinhead size or smaller, discrete, translucent, of a bluish, skim-milk color, was streaked on half of a second plate. From this a colony was streaked on Loeffler's blood serum and after from 16-18 hours' incubation was examined for morphology.

*Fermentation Reactions.*—Pure cultures were inoculated into tubes of 10 c c of Hiss' serum water to which ½ c c of a 20% sugar solution, sterilized fractionally, had been added. One-tenth of 1 c c of sterile Andrade indicator was added on the 2nd day. The tubes were read after 2, 4, 6 and 8 days of incubation. On the 8th day smears were examined for contamination.

It has been the experience of one of us in a study of diphtherial infection in wounds that the saccharose fermentation was of considerable importance as regards virulence, that is, that nonsaccharose fermenters are virulent and most saccharose fermenters are avirulent or only slightly virulent. Because of the difficulty in obtaining guinea-pigs and the unsatisfactory results obtained in intracutaneous virulence tests, only five cultures were tested. As chart 4 shows, two of the virulent strains, killing in 24 and 36 hours, respectively, were nonsaccharose fermenters, one being morphologically typical diphtheria, the other showing only wedge forms. The third culture which showed only wedge forms in original throat culture killed in 4½ days, and fermented saccharose. The other two strains were pure cultures of the atypical organism and had not killed at the end of 2 weeks. All control pigs were negative.

*Technic of Virulence Tests.*—Forty-eight hour infusion broth cultures were used. One-half of 1% of the body weight of each pig reckoned in c c was injected subcutaneously. A proportional amount of each culture was injected subcutaneously into a control pig which had been immunized 24 hours previously by an intraperitoneal injection of 100 units of antitoxin. From the pigs that died, cultures were made from the subcutaneous tissues at the site of injection, from the peritoneal fluid, and from the heart blood. The organism injected was recovered only from the one dying after 4½ days. The adrenals were congested in all three cases.

TABLE 1

| | Morphology | | Fermentation Reactions | | | | | | | | | | | | | | | | | | | | Virulence for Guinea-Pigs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Throat Culture | Pure Culture | Saccharose | | | | Dextrin | | | | Dextrose | | | | Lactose | | | | Maltose | | | | |
| | | | Days | | | | Days | | | | Days | | | | Days | | | | Days | | | | |
| | | | 2 | 4 | 6 | 8 | 2 | 4 | 6 | 8 | 2 | 4 | 6 | 8 | 2 | 4 | 6 | 8 | 2 | 4 | 6 | 8 | |
| B Clinical | Practically pure culture $D^2$ | $A_1B_1C_1$ Rare B & C | + | + | + | + | + | + | + | + | + | + | + | + | – | – | – | – | + | + | + | + | |
| E Clinical | B C | B C $A_1B_1C_1$ | – | – | – | – | ± | + | + | + | + | + | + | + | – | + | + | + | – | + | + | + | Test pig died 36 hr. Control pig, negative. Necropsy cultures, negative |
| S Clinical | $D_2$ Rare $C_1$ $D_2$ | $A_1B_1C_1$ Rare B C D | + | + | + | + | – | – | – | – | – | + | + | + | + | + | + | + | – | + | + | + | Test pig died 4½ days. Control pig, neg. Organisms in cultures of skin and blood |
| $S^1$ Culture from guinea-pig | | B C $B_1B_2$ $C_1C_2$ | – | + | + | + | – | – | – | – | – | + | + | + | – | – | + | + | | | | | |
| T Clinical | $B_1$ $C_1$ Rare B C | B C $A_1$ | – | – | – | – | – | + | + | + | – | + | + | + | – | + | + | + | – | + | + | ± | |
| G Clinical | B C | B C $B_1$ $C_1$ | – | – | – | – | – | + | + | – | ± | + | + | + | – | – | – | – | ± | + | + | + | |
| W Clinical | B C | B C $B_1$ $C_1$ | – | – | – | – | – | + | + | + | – | + | + | + | – | + | + | + | ± | + | + | + | |
| W. Y. Carrier | $D_2$ | $C_1$ $D_1$ $C_2$ $D_2$ | – | – | – | – | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | Test pig died 24 hr. Control pig, negative. Necropsy cultures, negative |
| W. R. Carrier | $D_2$ | $D_2$ | – | – | – | – | – | + | + | + | – | + | + | + | – | + | + | + | – | ± | – | – | |
| A Carrier | $D_2$ | $D_2$ | – | + | + | + | – | – | – | – | – | + | + | + | + | + | + | + | – | – | ± | + | |
| K Carrier | $D_2$ | $D_2$ Rare $C_1$ $D_1$ | + | + | + | ± | – | – | – | – | + | + | + | + | + | + | + | + | + | + | + | + | Test and control pigs negative |
| C Carrier | $D_2$ | $D_2$ Rare $C_2$ | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | |
| P Carrier | $D_1$ $D_2$ Rare D | $C_2$ $D_2$ | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | Morphology recorded according to Westbrook's classification |
| E. K. Carrier | B C | A B C $A_1B_1C_1$ | – | – | – | – | – | + | + | + | – | + | + | + | – | + | + | + | ± | + | + | + | |
| W. A. Carrier | B C | B C | + | + | + | – | – | + | + | + | + | + | + | + | – | – | – | – | | | | | Test and control pigs negative |

Table 1.—The fermentation reactions are different from those commonly given, in that B. and W. A. ferment both saccharose and dextrin, and ten of the cultures ferment lactose. The two most virulent strains, E. and W. Y. do not ferment saccharose; the less virulent, S., and avirulent, K. and W. A., do not ferment saccharose. Cultures C. and P. are morphologically and culturally B. Hoffmanni. Note that the virulent cultures S. and W. Y. show the wedge form (D-2) predominatingly or in pure cultures in the original throat cultures, while K. was avirulent. Cultures E. and W. A., both typically B. diphtheriae, were virulent and avirulent respectively.

The other data bearing on the virulence of this atypical organism were for the most part clinical. Chart 4 A gives a summary of our laboratory records up to Dec. 10. Of 434 cases in which the atypical organism was found, only 113 showed it on the following day, that is, in 75% of the cases it was only a transient inhabitant of the throat. Of the eighteen clinical cases showing both typical and atypical organisms, ten, or 11.1%, of all the clinical cases showed the atypical organism first and later the typical. In eight cases the reverse was true. It cannot be denied that in the ten cases the appearance of the typical form later can be explained by a fresh infection with this organism, since these cases were in the clinical diphtheria ward where cross infection was possible. But in these ten cases the atypical form was able to produce symptoms sufficiently suggestive of diphtheria to warrant their being admitted to the clinical rather than to the carrier ward, and we have had frequent evidence that at times this wedge form is one of the many forms that may be assumed by the extremely pleomorphic typical diphtheria bacillus.

On Dec. 6, 7 and 8 (chart 1), base hospital 49 showed a sudden increase in the number of atypical forms—one ward having 30. At the same time base hospital 26 showed a similar increase. On inquiry at the latter hospital it was found that the "team" taking the cultures had become somewhat discouraged at the large amount of work without apparent results, and had decided that it was time for the ward to come out of quarantine. With this end in view they gave up the use of tongue depressors and cultured at random about the tongue and teeth and in the anterior nares. The result was that all three wards cultured on that day showed from 15-25 cases of "Diagnosis reserved." One of these wards, cultured properly, had been entirely negative the day before. No such explanation could be found, however, in the case of base hospital 49. Morphologically this organism found in the mouth and anterior nares could not be distinguished from the wedge form we had been finding in the throat. Also the diphtheroid found on hands and fomites in wards where there had been no diphtheria, had the same morphology.

It is our opinion that cases showing this atypical organism in properly taken nose and throat cultures must be treated as positive diphtheria cases or carriers until each strain has been proved nonvirulent, since (1) two pure cultures of this organism killed guinea-pigs in 24 and 108 hours, respectively; (2) 11.1% of the clinical cases showed this form only at first and later showed the typical organism, and (3) variation in length of incubation may show first the atypical and then the typical forms. However, in the majority of cases, this form was only a transient inhabitant of the throat and, we believe, was avirulent.

## METHODS OF CONTROL

From the first, base hospital 56 was chosen as the contagious hospital for the Center and both clinical cases and carriers were sent there. The wards in which these were discovered were quarantined and cultured as directed by the head of the medical service in each hospital. It was early seen that much would be gained both in uniformity of results and in the availability of statistics by having the cultures examined at the central laboratory for the Center rather than by the several unit laboratories. Up to the first of Dec. all the cultures were examined by one man. Since then, owing to the increased number, it was found necessary to have two examiners who constantly consulted with each other on doubtful cases.

Toward the latter part of Nov., when from 1-7 new cases developed each day, it was felt that the methods of control at the various hospital units should be more rigorous and more uniform. It was decided to include as clinical cases all those with positive cultures and throat symptoms, though such symptoms might be very slight. Three methods of control were emphasized, any one of which by itself should, theoretically, control spread of infection.

1. *Individual Quarantine.*—The face[7, 8] mask epitomized individual quarantine. The quarantine of the ward from communication with the outside seemed to be fairly well understood and carried out, but the quarantine of the individuals within the ward from each other was almost completely neglected at that time. For instance, on the discovery of a clinical case the ward was isolated and cultured. Usually one or more positive carriers were discovered and reported in 24 hours. But during this 24 hours the patients who were up would congregate around the stove, owing to inclement weather, playing cards, waiting on the bed patients, and so on. The carriers who were about to be detected had been infecting others by droplet and hand. In other words, at the time when the ward culture was received at the ward it gave no accurate idea as to the number of individuals carrying the diphtheria bacilli in their throats. The same was true of the report on the second ward culture, and so one, ad infinitum. One ward was cultured four times in 7 days. The first culture showed 1 carrier; the second, 2; the third, 2, and the fourth, 4. After each report the carriers were evacuated to the contagious hospital, and it seemed to be only a question of time and endurance before the entire ward would be transferred.

The mask[9, 10] was suggested as a remedy. It was, of course, impracticable to mask all of the 16,000 individuals at the Center, though one hospital tried masking its entire personnel. The dissociation of mask from quarantine was found detrimental to the discipline and mental attitude, which is not the least important function of the mask. This might be termed "mask psychology." Cubicles were recommended to supplement the mask, since few can sleep with the

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[7] Capps, J. A.: Jour. Am. Med. Assn., 1918, 70, p. 910.

[8] Weaver, G. H.: Ibid., 1918, 70, p. 76.

[9] Doust, B. C., and Lyon, A. H.: Jour. Am. Med. Assn., 1918, 71, p. 1216.

[10] Haller, D. A., and Colwell, R. C.: Ibid., 1918, 71, p. 1213.

mask in place. It was urged that the patients be kept by their beds as much as possible, particularly during meals, when, of course, the mask must be removed. The original masks had two layers of gauze with a mesh 14 by 11 threads per cm. It was recommended that two such masks be worn, and that all future masks be made with four layers. Our first point, then, was individual quarantine as represented by mask and cubicle.

2. *The Schick Reaction.*—Theoretically, if we had examined every individual at the Center by the Schick test and immunized all positives with a prophylactic dose of antitoxin, the spread of diphtheria would have stopped at once. The detection of carriers would have been of no interest, since there would have been no one for them to infect. But this was not considered, because for some time it was questionable whether enough toxin was available for the quarantined wards alone, as had been recommended. A control of inactivated toxin was quite out of the question. The value of the Schick reaction without such a control was the subject of considerable discussion. It was felt that since our object was to stop the spread of the disease at this Center and not to obtain data as to the value of the Schick test, that by reading the reaction at 24, 48 and 72 hour intervals we could divide the reactions into three classes: Those frankly positive; those frankly pseudopositive, and those that were questionable. By immunizing cases showing a positive or a questionable reaction, we were erring on the side of safety. On 1,363 Schick tests reported from five of the hospitals, the percentages of positives were as follows: 8% at three hospitals, 6% at one, and 0.6% at another. Obviously, there was a flaw in the technic of the test at the last named hospital. There were a few carriers showing positive Schick tests, but shortage of guinea-pigs prevented our testing their cultures for virulence. Also an occasional patient having a negative Schick test came down with diphtheria a few weeks later. The explanations for this are either faulty technic, inert toxin, or overwhelming infection. Our second method of attack, then, was the Schick reaction plus prophylactic immunization.

3. *Nose and Throat Cultures.*—One of the earliest suggestions for the control of diphtheria was that all of the 16,000 individuals be cultured. Because of our limited autoclave space our daily output of medium could not be pushed beyond 2,000 tubes. There was also a shortage in personnel, both officers and enlisted men. It would, there-

fore, have taken 8 days at the very least, to culture the entire Center. Thus we would have had the same difficulties which we experienced in the wards before individual quarantine was instituted, only multiplied 8 times, since it was out of the question that during this entire period each individual at the Center would refrain from all except "masked" communication with all the others. At the end of 8 days, our summary report on the names and number of carriers at the Center would have no value, since during the 24-hour interval between culture and reports, each "positive" would have infected an unknown number of other individuals. So this plan was fortunately abandoned. Definite rules as to the number of times a ward was to be cultured following each new case were formulated. Emphasis was laid on a short, rigorous quarantine since it was felt that the effectiveness of a quarantine is in inverse ratio to its length. Personal cooperation of the patients is absolutely essential to individual quarantine, and this would be entirely lost unless they understood what is being done, why it is being done, and that the hope of release is reasonably within sight.

These rules were helpful not only in standardizing quarantine, but also in stemming the flood of cultures. There is always the danger that wholesale, promiscuous and nonselective culturing may give a false sense of security. For a period of 10 days — from Nov. 29 to Dec. 9 — 600 to 1,300 cultures were examined daily. The positives each day varied from 1.1-3.1% of the total cultures examined. The last 4 days of this period, when the percentage rose as high as 3.1% a large number of atypical forms was being found, partly accounted for by the careless mouth and anterior nares cultures previously mentioned. During the first six days the percentage varied only from 1.1-1.8%. If from this be subtracted the 0.3-0.6% of clinical cases each day, there is left a daily quota of carriers amounting roughly to 1% of those examined. This is what Rosenau[11] gives as the normal percentage of diphtheria carriers among the unexposed population. In other words, what we really had here was not a potential epidemic of diphtheria but an actual epidemic of culturing. Our third point in control is selective nose and throat cultures, properly taken.

Finally, the most important measure in the suppression of any epidemic is early diagnosis. This is of special significance in a disease such as diphtheria, in which the spread by contact is unquestionable, and, in addition, early diagnosis spells practically 100% cures.

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[11] Preventive Medicine and Hygiene, 1918, p. 1374.

The influence of delayed diagnosis on spread has already been discussed. To emphasize this, a special order was issued that the throats of all patients and personnel be inspected daily. All suspicious cases were to be isolated and cultured. Observation wards were established where such patients were held pending the report on their cultures. Such wards were strictly cubicled and masked, thus reducing contact infection to a minimum.

## CONCLUSIONS

The two probable modes of introduction of the diphtheria bacillus into this Center were "gassed" carriers and chronic carriers.

Factors in spread were crowding (direct droplet infection) contaminated hands and fomites (indirect droplet infection), and delayed diagnosis, partly explained by the similarity in symptoms between laryngeal diphtheria and membranous laryngitis due to gassing. Of these we consider crowding the most important.

Morphology of the diphtheria group is no criterion of virulence. Occasionally, difficulty was encountered in distinguishing between polar-staining, nondiphtheria bacilli and typical B. diphtheriae on morphology alone. Such confusion was experienced only in cultures from hands and fomites, but never in throat cultures.

The four methods of control employed were:

1. Early diagnosis through frequent throat examinations.
2. Individual quarantine exemplified by the mask and cubicle.
3. The Schick test followed by immunization of susceptible individuals with antitoxin.
4. Selective culturing of nose and throat carefully performed.

# NECROPSY STUDIES AT A HOSPITAL CENTER *

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## INTRODUCTION

The study in this paper covers a vast amount of material examined in a relatively short period of time. It is therefore more extensive than intensive. It is not possible to give an exhaustive discussion of all the lesions encountered—for that would involve little short of an unabridged treatise on pathology—but rather to give a brief and hurried résumé of all of the important pathologic conditions that came under observation. In the period under discussion, more than 30,000 patients passed through the center. At one time more than 20,000 patients and personnel were quartered here. The heavy pressure of work from all sides, together with the absence of proper library facilities, makes it impossible to give much more than a cursory sketch of the grosser details of the postmortem findings.

During the period included between July 23, 1918—when the first convoy of patients arrived at this center—and Jan. 1, 1919, there are recorded a total of 429 deaths. Of this number, about fifteen bodies were not necropsied for reasons other than the lack of time; these dead included nurses, officers from the personnel, allied soldiers, several bodies brought in crushed in railroad accidents, and one case of drowning among the enlisted personnel. The number of necropsies performed during this period was 356, or about 86 per cent. of the total possible number. This percentage is not inconsiderable when one observes the constant and progressive rise in the curve of the number of patients in the hospital during October and the early part of November. The shortage of personnel became very acute and presented itself in the most accentuated form in the laboratory services.

The highest death rate occurred during October, when 245, or considerably more than one half of the total number of deaths occurred. During this month the deaths averaged eight per day, with three deaths as the minimum and fourteen deaths as the maximum. Of these, the majority were due to respiratory diseases. Indeed, the total number of nonwounded men among the 356 cases that came to necropsy was 214, or in excess of 60 per cent.

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The important findings will be discussed in the order of their frequency. (See appended statistical résumé.)

### EMPYEMA AND PLEURISY

The most frequent necropsy finding in the entire series was empyema and pleurisy. This occurred in 63.2 per cent. of the series. The degree of pleurisy varied from a few cubic centimeters of serofibrinous exudate to several liters of thick pus. In most of the cases there were fibrinous deposits, occasionally large, shaggy, fibrinopurulent masses. The distinction between empyema and plain pleurisy was at times made only arbitrarily, depending on the quantity and character of the exudate. Real thick creamy pus was of rare occurrence, being present in only about twenty of the cases. The remaining thirty cases classified as empyema were characterized more by a thin, only slightly turbid, serous exudate, containing large fibrinopurulent masses. The abundance of this plastic, shaggy exudate was very striking. It was not at all unusual to find a yellowish-fibrinous deposit from 3 to 4 c.c. in thickness, covering the lower lobes of the lungs. Rarely was the exudate blood tinged. A number of the true empyemas were associated with gunshot wounds of the chest.

Of the "nontraumatic" empyemas, only about 50 per cent. of the cases were diagnosed clinically. In a few cases in which thoracostomy was performed, the drainage tubes were found inserted 2 to 4 inches into the chest cavity, so that from 500 to 1,000 c.c. of pus were still undrained.

Subacute or chronic pleurisy was encountered fifty times, or in 14 per cent. of the cases. A combination of both chronic and acute pleurisy existed in thirty cases, or 8.5 per cent.

The terms "chronic" and "acute" are often misnomers. We term lesions with fibrous adhesions as chronic. Yet these may be of only short duration. A case came to necropsy which demonstrated how quickly these adhesions may arise. A man was wounded by a piece of shrapnel in the chest and was sent to the hospital with a diagnosis of a foreign body in the lung as revealed by the roentgen ray. In the hospital the roentgen ray confirmed the diagnosis until about one day before death, which was twenty-two days after the injury. A roentgenogram taken at this time in a changed position of the patient showed the bullet movable in the pleural cavity. At necropsy there was found a localized empyema in the pus of which was present a small piece of shrapnel. The adhesions walling off this empyema were exceedingly strong and along the entire margin of the "abscess" cavity there were dense, hyalinized, grayish white, connective tissue adhesions, 3 mm. in thickness, which abruptly thinned out over the uninvolved portions of the lung and pleura. The involved portion of the pleural cavity occupied about two thirds of the space on that side; the rest was entirely normal. The other pleural cavity was also free from lesions. Here, then, was a lesion with dense fibrous connective tissue formation, 3 mm. in thickness, which formed during the short period of twenty-three days.

### PNEUMONIA

The next most frequent finding was pneumonia. This condition was present in 218, or 61.2 per cent. of the cases. The intensity of the involvement varied from almost miliary areas of consolidation around the bronchioles, to complete hepatization of entire lobes on one or both sides. Bronchopneumonias were most common, constituting more than three fourths of the cases. These at times were so massive and confluent that they resembled true lobar pneumonias. Some of the cases diagnosed at necropsy as lobar pneumonia, which later proved to be streptococcic infections culturally, were probably of this type.

Cultures and smears from the lungs were taken frequently. The cultures were made by searing the cut surface thoroughly and then, with a glass pipet having a rather large opening, a small quantity of macerated pulp, mixed with expressed fluid, was drawn up and inoculated into appropriate media. The maceration was accomplished by working the end of the pipet up and down into the parenchyma through the sterilized surface.

The frank characteristic lobar pneumonias in the gray hepatization or early resolution stage, as found ordinarily, were very uncommon. Not more than twenty such cases were encountered. The other cases classified under lobar pneumonia were atypical. An entire lobe or even an entire lung was often involved, but this involvement was irregular. The organ, though solid and heavy, was not large, and the cut surfaces were very mottled showing grayish, brownish or dark red areas of hemorrhage and edema with small portions that would still float in water. Areas of necrosis with abscess formation were quite frequent.

One type of pneumonia was encountered that was different from all ordinary pneumonias. In this type the involvement was either of an entire lobe or only of portions of one or several lobes. The involved areas were firm, heavy and had a rubber resiliency. The cut surfaces were dark red or dark brown in color, often showing a mottling with miliary or submiliary yellowish areas. Sometimes there was a fine grayish lace work visible through the reddish parenchyma that suggested thickened alveolar walls. These areas seemed to be most prominent along the bronchial branches. Culturally this type of pneumonia gave invariably a streptococcus, either hemolytic or nonhemolytic.

Microscopically, in the earlier stages, the alveolar walls show a thickening and an infiltration with lymphoid and plasma cells. The alveoli contain serum, red blood cells, a few polymorphonuclear leukocytes and many desquamated epithelial cells. The walls are edematous and the capillaries are dilated. Some show an intense infiltration with polymorphonuclears. In the later stages, the alveolar walls are seen invading the fibrinous plugs in the alveoli. The alveolar walls themselves appear like dense cellular bands of connective tissue, infiltrated with lymphocytes. Some areas show an almost complete fibrosis with practically all of the alveolar structure absent. Here and there may be seen alveoli lined with high cuboidal or even columnar epithelium suggesting attempts at regeneration. The last stage of this pneumonia is identical with the typical chronic, organizing or indurative pneumonias of the textbooks.

The histories in these cases were not at all characteristic, and did not even suggest the type of lesion present. Some cases had a rather acute onset with a history of not much more than a week's duration. Others presented histories extending over two, three or sometimes four weeks.

The frequent gradations from one type of pneumonia to another and the occasional mixture of all the types in a single lung, made it very difficult at times to classify properly the lesions encountered. Histologic study has occasionally demonstrated the existence of interstitial pneumonia where no such diagnosis was made at necropsy grossly; and conversely some cases diagnosed as interstitial pneumonia proved to be only a massive bronchopneumonia with extensive edema and hemorrhage accompanied by very little purulent exudate.

## HEART'S BLOOD CULTURES

Blood cultures were taken routinely at all necropsies. The anterior surface of the right ventricle was sterilized with a pronged searing iron and about 5 c.c. of blood removed with a sterile glass pipet. This was inoculated into two or three tubes of dextrose broth. Sometimes a portion of this mixture was plated out in the laboratory. Pneumococci were tested for bile solubility.

The number of positive cultures was high, about 50 per cent. This is considerably higher than in civil life experience. Of this number more than one half showed *S. hemolyticus.* Not a single case of a true *S. viridans* was obtained. The one pure culture of this organism identified came from a blood culture taken a few days before death in a case of septicemia.

*B. welchii* was recovered through blood culture from several cases in which no gaseous gangrene was present, and from two patients who were not even wounded. The *B. pyocyaneus* was recovered twice in pure culture and three times mixed with other organisms.

## GUNSHOT WOUNDS

About 40 per cent. of the cases in this group came to necropsy. This included minor as well as major injuries. In a number of these cases, the wounds had nothing to do with the actual cause of death. The extremities suffered the largest number of injuries. Fractures of the femur and the ilium contributed to death most frequently outside of the direct injuries to the peritoneal cavities which resulted in general peritonitis. Under injuries to the chest were included injuries to the dorsal spine as well as to the thoracic cavity. Every case of spinal cord injury resulted in extensive gangrenous and ulcerative cystitis.

Striking features in the cases of head injuries were the finding of massive destruction of brain tissue in cases up to six weeks after the inception of the injury. More than once, the question that arose in the mind of the pathologist was not why did the patient die or what caused his death, but why did he or how could he live so long?

In many of the gunshot injuries the infection was very great, causing dissecting abscesses to extend up and down the tissues from the point of injury. Sometimes several square feet of skin and muscle tissue were undermined by the spreading infection. In many cases, at the time of necropsy, it appeared that the surgical treatment was altogether too conservative. Often the debridement was not extensive enough, while in other cases a limb was saved at the expense of a life.

## OTITIS MEDIA AND SINUSITIS

Very early in the postmortem examinations the presence of pus in the sinuses of the skull was a striking feature. It was most constantly present in cases in which pneumonia was the prominent lesion. When only one sinus was involved, it was usually the sphenoid. One fourth of all the cases showed purulent sphenoidal sinusitis and frequently the pus "welled" up, as soon as the sinus was opened. Otitis media was relatively infrequent and when present contained exudate in a much lesser degree.

Smears and cultures from the pus frequently showed no organisms. When organisms were present they usually corresponded to those found in the cultures from the heart's blood or from the lung. Streptococci and pneumococci were therefore the most common bacteria. The *B. influenza* of Pfeiffer was exceedingly rare, being found in only one case of sphenoidal sinusitis.

The question of etiology is an interesting one. Were the bronchopneumonias, so frequently encountered, secondary to the sinusites, or were the sinusites secondary to the pneumonias? The possibility of a "reversible" reaction has to be admitted. Infected material from the sinuses might drain down the pharynx and through the larynx gain entrance to the terminal bronchioles where it might set up a purulent bronchitis and a subsequent pneumonia. But the reverse may also occur. A patient in bed with bronchopneumonia cannot help getting the nasopharynx bathed in sputum from whence infection may spread to the sphenoidal and ethmoidal sinuses. Our necropsy material did not aid in clarifying this relationship, since priority in either lesion could not be established. On a few occasions it was thought that the sinusites appeared to be the older lesions.

## INFLUENZA

This is not a finding at necropsy; but under this heading are discussed the lesions found at necropsy in eighty-seven cases in which the diagnosis of influenza was present on the field medical card as the cause of the patient's admission to hospitalization. Probably some of these cases were diagnosed wrongly; perhaps some patients died as a result of complications of influenza in which the proper initial diagnosis was not entered.

Of the eighty-seven cases so diagnosed, 86.2 per cent. showed complicating pneumonias. These pneumonias were not greatly dissimilar to other pneumonias except in a few details. First, the percentages of lobar and interstitial types are higher in this group than in the total pneumonias. This may be accounted for by the fact that in these cases the pneumonias played a more important rôle in the causation of death than in the others, since in the total pneumonias many of them were only terminal conditions, secondary to severe wounds, typhoid fever, etc. The percentage of identified pneumococcus pneumonias is also higher.

Abscesses were more frequent. Dilatations of the bronchioles and bronchi were fairly common. Some of the sacculations measured several centimeters in diameter. Some structures that appeared like abscesses were proved on closer observation to be bronchiectases of the saccular type.

Parallel with the high percentage of pneumonias ran the high percentage of sinus involvement. Fifty out of the eighty-seven cases showed this condition.

Meningitis was found in 8 per cent. of the cases admitted as influenza. One of these was a tuberculous case. This may have been a mistaken initial diagnosis, since the early symptoms of the tuberculous meningitis might have simulated influenza. The two cases of pneumococcic meningitis were secondary to pneumonia and empyema, which in turn were probably secondary to influenza. With the four cases of meningococcic meningitis it is difficult to decide whether the influenza actually antedated the meningococcic infection or whether there was a mistaken initial diagnosis. It has been stated that influenza may increase the susceptibility to meningeal infection, especially meningococcic.

One of the negative findings is worth mentioning—the almost complete absence of recently activated tuberculous lesions by preexisting influenza, a complication frequently mentioned in the textbooks. Reference to the attached statistical résumé will show that the percentage of occurrence of this lesion in the cases of influenza was less than half of that found in the total number of necropsies.

The other associated lesions, such as Zenker's degeneration, septicemia, pericarditis, etc., need no special discussion here. Their frefrequency can be found by referring to the statistical summary accompanying this paper.

A few bacteriologic reflections may be relevant at this point. A careful study of the material, both from the pathologic appearances and from the bacteriologic findings, suggests that the influenza bacillus of Pfeiffer did not play an important rôle in the cases of influenza treated at this center. Out of a total number of 356 necropsies, only three cases revealed a bacillus in cultures or in smears which morphologically and culturally resembled the *B. influenzae*. This was found in one case in smears and cultures of the lung; in another, in smears and cultures from the sphenoidal sinus, and in a third, in cultures from the blood. In these three cases the organisms were present not in pure culture but mixed with pneumococci. Although in a large majority of the cultures made at necropsy, the conditions were favorable for the growth of the influenza bacillus, nevertheless this organism revealed itself very rarely. Neither was it found in direct smears from the pus in the sinuses nor from the lungs. It might be argued that the cases which came to this center were somewhat late in the stage of the disease, and that this fact might explain the absence of the true causative agent and the presence of the secondary invaders. But a casual study of the literature on this subject tends to show that similar findings are not uncommon in many of the laboratories throughout the world. Careful bacteriological studies in some of the camps of the United States have almost convinced the investigators that the *B. influenzae* plays a very unimportant rôle, if any, in the etiology of influenza.

Considering the method of spread, the kind of complications and the character of the lesions found at necropsy, influenza may be considered as a highly contagious, but self-limited disease of unknown etiology, which bears a remarkable resemblance to measles. Indeed, it is convenient to look on influenza as nonexanthematous measles. There are several reasons for such a concept; the extreme contagiousness of both diseases associated with coryza, pharyngitis and lacrimation; the inability to demonstrate definitely the causative agent; the relative absence of leukocytosis, which is especially striking as the onset of the complicating pneumonias; the frequency of the pneumonic complications which are nearly always the cause of death in these diseases; the gross and microscopic appearance of the lesions in the lungs, bronchi and bronchioles with the occasional production of interstitial pneumonias and the frequent bronchiectases. It is common knowledge that one of the important etiologic factors of bronchiectasis is measles. And those who perform necropsies in cases with antecedent influenza histories are forcibly impressed with the frequency of the irregular fusiform and saccular dilatations of the bronchi and bronchioles. (In this connection it will be interesting to follow up the cases of influenza leading to convalescence and see the frequency of chronic bronchiectases that will reveal themselves later.) Just as measles predisposes the individual to secondary invasions of the respiratory tract, as a result of pathologic changes occurring in the epithelium of the tracheobronchial tree, so also it would seem that influenza produces not unlike changes which greatly facilitate secondary invasion by organisms such as the streptococcus, pneumococcus or *B. influenzae*. It seems reasonable to assume that the type of invasion depends to a great extent on the type of organism present in the throats of large numbers of individuals in any given locality. In places where the influenza bacillus is prevalent, this organism will be found in the associated inflammatory conditions. In districts where a certain type of pneumococcus is present, the pneumococcus will be found as the principal invader. In areas where the hemolytic streptococcus is the inhabitant of the throat in a large percentage of the patients, the hemolytic streptococcus will be found in the lungs, pleural cavities and other organs or tissues of the body.

While carrying on investigations in this laboratory on the spread of diphtheria by means of the hands and fomites,[1] the results suggest that the rapid spread of influenza may, to a very large degree, be due to extensive contamination of the hands and fomites with the virus. This contamination is due to the fact that nearly all the cases of influenza present more or less pharyngeal involvement and coryza; conditions which necessitate the frequent use of handkerchiefs, and these consequently soon become saturated with the secretions. Thus the hands of the patients remain persistently contaminated—a condition which even the rigid discipline of the doctors and nurses cannot prevent. There is practically no disease, with the exception of the "common colds," which has a greater chance of perpetuating an almost continuous state of contamination of the hands than does influenza during the early, acute stage of the disease.

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[1] Barron, M., and Bigelow, G. H.: Diphtheria at a Hospital Center, J. Infect. Dis. **25**:58 (July) 1919.

The study of influenza at this center emphasizes the fact that the etiology of this disease is as yet unknown. The great diversity of findings in different laboratories throughout the world makes it reasonable to assume that a filtrable virus is most probably the etiologic factor. This organism, whatever it may be, would produce in most cases only a self-limited disease were it not complicated by the secondary invaders such as *Streptococcus hemolyticus*, pneumococcus or *B. influenzae*. This invasion is consequent to a lowering of the barrier in the epithelium of the respiratory tract by this unknown agent. The disease is spread by nasal and pharyngeal secretions and probably also through the conjunctivae. Hands and fomites probably play a very important rôle. It would seem that from a clinical standpoint effort should be directed, for the present at least, not so much to prevent the spread of influenza, which is so difficult, but to prevent the entrance of the secondary invaders which nearly always are responsible for the seriousness of the disease and for death, when the latter occurs.

## NEPHRITIS

Acute parenchymatous changes of the kidneys were relatively frequent, occurring in about one fifth of the cases. However, about one half of these cases were very mild. Fairly definite lesions were present in about 11 per cent. of all cases. No characteristic gross kidney lesions were noted in cases of uncomplicated gaseous gangrene. Lesions sufficiently pronounced to cause death were encountered only twice, or in 0.6 per cent. It was gratifying to note how very few definitely chronic lesions of any kind had escaped the observation of the Army examining boards when recruiting was in progress.

## PERITONITIS

Peritonitis was encountered forty-one times. Of the thirty-seven cases of acute peritonitis, twelve were attributable directly to gunshot wounds. Several of these patients were definitely not transportable and died soon after their admission to the hospital. One case of peritonitis had an unusual and obscure etiology. There was found a complete rupture of the ileum a short distance above the ileocecal valve. The intestine was completely severed as if by a pair of scissors. The ends were black and necrotic for a distance of a few millimeters. The healthy portion of the intestinal wall stopped abruptly. The mesentery opposite the rupture was thickened and indurated. On the superior surface, at a point 2 cm. above the intestinal attachment, there was an ulcer, 15 mm. in diameter and 5 mm. in depth, involving a lymph node. The base and walls were necrotic and covered with purulent exudate. The upper surface of the intestinal coil had curled upward, plastering itself firmly to the mesentery and covering the lower margin of the ulcer. It apparently was Nature's vain attempt to prevent the rupture of the suppurating lymph node into the peritoneal cavity. On the inferior surface of the mesentery opposite this ulcer there were strong fibrous adhesions binding the mesentery to the brim of the pelvis. The rupture of the intestine probably resulted from this inflammation and ulceration, completely cutting off the blood supply of one small segment with the resulting rupture. The cause of the suppuration of the lymph node could not be determined.

## GAS INHALATION

Of the patients who died, 10.9 per cent. gave a history of gassing. Nearly all were cases of mustard gas inhalation and contact. The pathology of these cases was studied in greater detail by a member of the Central Laboratory staff.[2] All gradations of lesions were found, from acute congestion of the mucosa covered by a thin fibrinopurulent exudate to that of a severe fibrinopurulent tracheobronchitis with ulceration. The most advanced cases presented numerous ulcers through the mucosa of the trachea and bronchi with extensive destruction of tracheal cartilages. There were large areas of gangrene with abscess formation in the lung. Nearly all of the advanced cases showed abscesses and gangrene of the lungs. The exudate in the larynx was generally very pronounced, and often diphtherial in appearance, so that on several occasions there resulted confusion in diagnosis.[1] The complicating pneumonia was always of the bronchogenic type. Hemorrhages into the lungs were common.

## PULMONARY TUBERCULOSIS

Of the thirty-six patients (10.1 per cent.) showing pulmonary tuberculosis, 66⅔ per cent. had chronic or healed lesions. These presented simply old scars or fibrosed nodules at the apices, most often on the right. Caseating and calcareous nodules were sometimes present. Of the twelve cases of acute, or acute and chronic, tuberculosis, only a few had lesions extensive enough to be factors in the final exitus. In practically no case could tuberculosis be assigned as the primary cause of death.

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2. Covey: Pathology of Mustard Gas Inhalation, Am. J. M. Sc. **157**:808 (June) 1919.

### SEPTICEMIA

Nine per cent. of all cases had gross evidences of septicemia at necropsy in conjunction with antemortem as well as postmortem positive blood cultures. Three fourths of the cases were due to *Streptococcus hemolyticus.* The most constant findings in these cases were extensive jaundice of the skin and conjunctivae, together with subepicardial and subpleural petechial hemorrhages.

### MENINGITIS

Altogether, twenty-two cases of meningitis came to necropsy. Exactly one half of these were meningococcic in origin. The mortality of this form of meningitis was very unsatisfactory in spite of the energetic treatment with large quantities of serum.[3] Three cases were due to pneumococci, two of which occurred with an initial history of influenza. All three cases were superimposed on pneumonias or empyemas. In one case, the spinal fluid was very turbid, and direct smears of the uncentrifuged specimen revealed the pneumococcus in numbers comparable to those found in twenty-four-hour cultures. Typing revealed the pneumococcus to belong to Group II.

There was one case of tuberculous meningitis. The diagnosis was made shortly before death by finding tubercle bacilli in the fluid. At necropsy, there was found, in addition to the advanced meningitis, tuberculosis of the kidneys and bladder and tuberculous caries of the eleventh dorsal vertebra. Nothing but a shell of the body of the vertebra remained. This was wholly unsuspected, since it had given no clinical symptoms.

The seven other cases in the series were mostly streptococcal, and were consequent to traumatic lesions of the brain and spinal cord.

### ANEMIA FROM HEMORRHAGE

Severe secondary anemias, sufficient to produce symptoms and death, were found in 4.5 per cent. of the cases. In a few of these, the anemias were complicated by septicemias. The majority of the uncontrollable hemorrhages followed ruptures, through gunshot wounds, of the femoral and gluteal blood vessels. One patient died of hemorrhage from the internal maxillary artery which continued to bleed even after the common carotid on that side had been ligated.

### GAS GANGRENE

Gas gangrene was present in fifteen cases. When the organism was identified, it nearly always proved to be the *B. welchii.* In no case was the *B. edematiens* recovered, although several of the cases presented gross lesions—very extensive edema with very little gas in the tissues—characteristic of the bacillus of toxic edema. In antemortem examinations of wound anaerobes, the *B. vibrion septique* was identified in five cultures. The brownish red, dry, very friable, crepitating muscles produced by the *B. welchii* was very characteristic and easily identified.

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[3] Bigelow: Nonepidemic Epidemic Meningitis, Arch. Int. Med. **23**:723 (June) 1919.

## PERICARDITIS

Pericarditis was present in 3.9 per cent. of the cases. In several cases there was present a purulent or fibrinopurulent exudate to the extent of a liter or more within the pericardial cavity. Yet, with perhaps one or two exceptions, no antemortem diagnoses of the condition were made. It doubtless proved, relatively at least, the greatest diagnostic pitfall.

The chronic pericarditides were of the adhesive type. The chronic "patchy" epicarditis—the so-called "soldier spots" of some writers—were very interesting because of their frequency. Unfortunately, they were so common, that not sufficient attention was paid to them. They were encountered many times more frequently than in civil life. Many stages in their development could be traced out. There were some with a patch about 2 cm. in diameter situated over the right ventricle anteriorly, several centimeters above the apex. From this patch, a bundle of white, coarse adhesions, 2 cm. in length, extended, uniting it with the corresponding surface of the pericardial wall. The next stage found was where the adhesions were already ruptured showing a large tuft of adhesions hanging free from the epicardium and a smaller tuft of adhesions projecting from the pericardial sac. The next stage showed a rough, thickened epicardial patch with no or only a few very short tags present. The corresponding surface on the pericardium showed slight evidences of adhesions or thickening. The last stage showed a simple, opaque, glistening, white, thickened patch of the epicardium. There were no more evidences of any lesions on the corresponding point of the epicardium.

## ENTEROCOLITIS

Twelve, or 3.3 per cent., of the cases showed lesions in the large and small intestines other than those resulting from typhoid fever. Of these twelve cases, three were due to *Endameba dysenteriae* in which the parasites were demonstrated in preparations made from the ulcers. Two cases presented classical pictures of bacillary dysentery. The involvement was principally in the colon, but extended also for a considerable distance up into the ileum. The crests of the folds of mucous membrane were covered with thick, dull layers of exudate. The mucosa was edematous and hemorrhagic. Numerous ulcers were present which in some areas were so extensive as to leave only islands of polypoid patches of mucous membrane. Cultures were taken from the feces and heart's blood, but not from the spleen or mesenteric lymph nodes, and this may account for the negative bacteriologic evidences for the identification of the lesions. Seven cases had extensive lesions in which neither endamebae nor *B. dysenteriae* were demonstrated, and the gross pictures of the ulcerations were not sufficiently characteristic to make a definite diagnosis. Not a single case of *B. dysenteriae* of any type was isolated at this center, although numerous cultures were made of suspected stools. Typhoid and paratyphoid organisms were occasionally isolated both from the feces and from the urine, as well as from necropsy material.

### ZENKER'S DEGENERATION

Parenchymatous degeneration of the recti muscles with hemorrhage and rupture was strikingly frequent. Several of the cases were frank cases of "Zenker's" degeneration, both grossly and microscopically. The others showed the swelling of the muscle fibers, the hemorrhage and rupture of the muscle bundles, but the yellowish "waxy" appearing areas were not so prominent grossly.

Seven of the nine cases were associated with pneumonia following a history of influenza. In one of these there was also present a hemorrhagic encephalitis. Of the two remaining cases, one was associated with pneumonia complicating gunshot wound, and the other with pneumonia in which the initial diagnosis was not stated. Not a single case of typhoid presented this lesion, although typhoid is considered in the textbooks as being the principal disease in which it is found.

Several interesting confusing diagnoses resulted from the hemorrhage and rupture of the abdominal rectus muscles. In one case a diagnosis of appendicitis was made which was fortunately treated medically and not surgically. At the necropsy the appendix was found to be normal, but the lower third of the right rectus muscle showed typical Zenker's degeneration with rupture. In another case, the patient suddenly developed signs of acute cholecystitis. All his symptoms were referred to the gallbladder region. There was marked tenderness, severe pain, some rigidity and a distinct mass was palpable. An area of dulness the size of an orange, in the region of the gallbladder, was outlined. The findings were so definite that it was decided to operate, but not until a careful consultation was held. A diagnosis of acute cholecystitis, probably suppurative, was made. On the day set for operation, the patient developed edema of the lungs and sank into a condition too serious for operation. He died several days later. At necropsy, the gallbladder was found to be entirely normal, and this time the upper right rectus muscle showed Zenker's degeneration with rupture and extensive hemorrhage into the sheaths which extended upward to the insertions at the costal cartilages. The greatest amount of blood clot was found just below the costal margin.

One point of differential diagnosis between these lesions in the rectus muscles and true surgical conditions within the peritoneal cavity was brought out at a discussion in one of the clinicopathologic conferences. Whereas tenderness and pain is elicited readily by very superficial palpation in the first instance, it is only on deeper palpation that the maximum discomfort is produced in the latter case.

## TYPHOID FEVER

In this series eight cases of typhoid fever were encountered. Only one or two were diagnosed clinically. The lesions in the intestines were characteristic, but extensive ulcerations in the colon were more frequently encountered than is the usual experience. In most of the cases, the *B. typhosus* was recovered from the spleen, gallbladder and the heart's blood. A few cases failed to give any bacteriologic evidences of the disease although the gross lesions were definitely diagnostic.

The clinical diagnosis of typhoid and paratyphoid fever proved quite a problem. In many cases at the center which were clinically typhoid, no corroborative laboratory evidences could be obtained either in positive blood cultures or in positive stool or urine cultures. The ordinary Widal test is, of course, worthless in prophylacticated individuals. One of the laboratory staff[4] obtained interesting results by using Dryer's quantitive agglutination, which promises to be a valuable aid in differential diagnosis of this disease in prophylacticated individuals, where cultural evidences are negative. No known case of paratyphoid fever came to necropsy.

## DIPHTHERIA

The mortality from diphtheria was very disappointing. Several patients dying of diphtheria came to necropsy with the case undiagnosed clinically. The reasons for this are discussed fully by Barron and Bigelow.[1] All cases were of the laryngeal type — a condition rather unusual for adults.

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[4] Eggerth: A Study of Dreyer's Agglutination Technique in the Army, J. Infect. Dis., 1919 (in press).

## HEMORRHAGIC ENCEPHALITIS

Three cases of hemorrhagic encephalitis are included in the series. One case was associated with ascending myelitis associated with complete transverse laceration of the lower dorsal cord following gunshot injury. Another case was associated with pneumonia following a history of influenza. The third case was associated with a beginning bronchopneumonia in a patient who had apparently completely recovered from a gunshot wound. The patient was ready for evacuation when he became sick very suddenly with a high temperature, rapid pulse and increased respiration. The next morning after his readmission to the hospital, a diagnosis of lobar pneumonia was made. Shortly afterward, he developed symptoms of increased intracranial pressure, with subnormal temperature, pulse 46, respiration 8. Retinoscopy revealed a "choked disc." He died less than forty-eight hours after admission. At necropsy there was extreme injection of the pia mater and the arachnoid membrane. Section of the brain showed the white matter peppered with pinpoint to pinhead sized hemorrhages most marked in the corpus striatum on the left side, the corpus callosum and in the pons. The lungs showed only the very earliest stage of bronchopneumonia. The blood culture made at necropsy was sterile. Unfortunately no cultures were made from the brain tissue.

## CONGENITAL ANOMALIES

Of the fourteen prominent congenital anomalies, exactly 50 per cent. involved the kidneys. There were three cases of horseshoe kidney. Two cases of hypoplasia were found in which the involved kidneys were about the size of lima beans, while the kidneys on the opposite side were about twice the normal size, a compensatory hyperplasia. There were two cases of complete aplasia of one of the kidneys. In these, not only was there no vestige of kidney substance found, but there was not a trace of the corresponding ureter.

Of the remaining anomalies, only one other is worthy of mention, that of an accessory lung. A mass of tissue entirely covered with pleura, having the size, shape, appearance and consistency of one of the lobes of a lung in a full term fetus, was found in the left pleural cavity attached by a narrow pedicle of connective tissue to the anterolateral prevertebral tissue in the region of the eighth dorsal vertebra. This pedicle appeared like the structure of the hilus of a lung, but had no relation to any of the structures of the normal lung. The only connection discovered between it and the normal structures was through two arteries, each about 2 mm. in diameter (outside), arising from the aorta in that region. These arteries were, of course, entirely anomalous. The parenchyma did not crepitate and contained no air. On section, a miniature bronchial tree could be identified in which the structure corresponding to a bronchus measuring about 5 mm. in diameter ended blindly and bluntly in the loose cellular tissue of the "hilus" near the two arteries. Microscopically, the tissue shows definite bronchial and bronchiolar structure, with cartilages, nonstriated muscle fibers and connective tissue. The blood vessels are prominent. The lumen is lined with a high columnar epithelium and contains mucoid material mixed with epithelial cells and other cellular debris. The parenchyma shows a rather loose cellular connective tissue through which are scattered islands of cuboidal and columnar epithelial cells, some thrown together irregularly, others arranged in gland-like structures. This tissue will probably be studied more in detail by me at some future time.

## SUMMARY

A number of important facts reveal themselves in the study of this material. A few of them are the following:

Empyemas and acute pericarditides are not so readily diagnosed as the textbooks would make one believe. These conditions have proven pitfalls for some of our best diagnosticians.

A very large percentage of the pneumonia cases have associated suppurative conditions in one or more of the sinuses in the skull. These present very meager, if any, clinical evidences, and nearly always remain undiagnosed until the postmortem examination.

Influenza practically never kills by itself alone. In the present series, pneumonia was the cause of death in nearly all of the cases, a few patients dying of meningitis, typhoid fever, etc. *B. influenzae* of Pfeiffer played no important rôle.

Of all the deaths, about 75 per cent. were medical. Although almost 40 per cent. of the deaths occurred in wounded persons; in a considerable proportion of these the wounds were far too slight to play any important part in causing death.

The surgical treatment of gunshot wounds was at times not sufficiently intensive. Cases came to necropsy which probably might have been saved by amputation of an involved extremity, or by more radical debridement.

Some surgical cases with gunshot injuries to the abdomen were evacuated to base hospitals while in a nontransportable condition.

Zenker's degeneration, with rupture and hemorrhage, is associated with highly virulent infections. It sometimes may cause confusion in diagnosis from intra-abdominal surgical conditions.

Congenital anomalies were present in about 4 per cent. of the cases, and one-half of these involved the kidneys.

Practically no well established chronic lesions escaped the detection of the recruiting medical examining boards.

## RÉSUMÉ OF THREE HUNDRED AND FIFTY-SIX NECROPSIES

### Statistical Data Up to Jan. 1, 1919

### GENERAL CONSIDERATIONS

| | |
|---|---|
| Total number of patients treated | 29,196 |
| Total number of deaths | 429 |
| Mortality rate | 1.47 per cent. |
| Total number of necropsies | 356 |

| | Patients Treated* | No. of Deaths | Mortality Rate, % | No. of Necropsies |
|---|---|---|---|---|
| Evacuation Hospital 19 | 4,951 | 7 | 0.14 | 7 |
| Base Hospital 26 | 5,512 | 99 | 1.79 | 85 |
| Base Hospital 25 | 5,860 | 117 | 1.99 | 97 |
| Base Hospital 49 | 4,626 | 84 | 1.81 | 76 |
| Base Hospital 56 | 7,766 | 82 | 1.05 | 67 |
| Base Hospital 70 | 5,371 | 40 | 0.78 | 24 |

| | |
|---|---|
| Date of first convoy of patients to center | July 23, 1918 |
| Date of first necropsy | July 26, 1918 |

### NECROPSY FINDINGS

| | |
|---|---|
| Empyema and pleurisy | 225 cases or 63.2% |
| Acute pleurisy | 95 cases or 26.7% |
| Empyema | 50 cases or 14.0% |
| Chronic pleurisy | 50 cases or 14.0% |
| Chronic and acute pleurisy | 30 cases or 8.5% |
| Pneumonia | 218 cases or 61.2% |
| Bronchopneumonia | 165 cases or 75.5% |
| Streptococcic | 64 cases or 38.8% |
| Pneumococcic | 20 cases or 12.1% |
| Undetermined | 81 cases or 49.1% |
| Lobar pneumonia | 37 cases or 17.0% |
| Pneumococcic | 16 cases or 43.2% |
| Streptococcic | 11 cases or 29.8% |
| Undetermined | 10 cases or 27.0% |
| Interstitial | 16 cases or 7.5% |
| Streptococcic | 16 cases or 100 % |
| Positive blood cultures | 163 cases or 45.8% |
| Hemolytic streptococcus | 84 times or 51.4% |
| Nonhemolytic streptococcus | 35 times or 21.3% |
| Pneumococcus | 26 times or 16.0% |
| B. welchii | 12 times or 7.3% |
| B. pyocyaneus | 5 times or 3.4% |
| Staphylococcus | 1 time or 0.6% |
| Number of wounded | 142 or 39.8% |
| Parts wounded | 163 |
| Extremities | 86 or 52.7% |
| Abdomen | 28 or 17.1% |
| Chest | 26 or 16.0% |
| Head | 23 or 14.2% |

---

* This total includes 4,890 patients received by transfer.

| | | |
|---|---|---|
| Number of bones fractured | 91 | |
| Ribs | 13 | |
| Femur | 12 | |
| Ilium | 10 | |
| Vertebrae | 10 | |
| Skull | 9 | |
| Tibia | 7 | |
| Fibula | 4 | |
| Scapula | 4 | |
| Humerus | 3 | |
| Coccix | 3 | |
| Radius | 3 | |
| Ulna | 3 | |
| Ischium | 3 | |
| Metacarpal | 2 | |
| Sacrum | 2 | |
| Ankle | 1 | |
| Clavicle | 1 | |
| Jaw | 1 | |
| Number of cases with otitis media and sinusitis | 120 | or 33.7% |
| Number of otitis media and sinusitis involvements | 192 | |
| Sphenoidal sinusitis | 92 times | or 25.8% |
| Frontal sinusitis | 34 times | or 9.5% |
| Ethmoidal sinusitis | 35 times | or 9.8% |
| Otitis media | 31 times | or 8.7% |
| Influenza | 87 cases | or 24.4% |
| Pneumonia following influenza | 75 cases | or 36.2% |
| Bronchopneumonia | 48 cases | or 64% |
| Streptococcic | 22 cases | or 45.8% |
| Pneumococcic | 14 cases | or 29.2% |
| Undetermined | 12 cases | or 25.0% |
| Lobar pneumonia | 18 cases | or 24% |
| Pneumococcic | 12 cases | or 67% |
| Streptococcic | 4 cases | or 22% |
| Undetermined | 2 cases | or 11% |
| Interstitial pneumonia | 9 cases | or 12% |
| Streptococcic | 9 cases | or 100 % |
| Number of cases with otitis media and sinusitis | 50 | or 57.4% |
| Number of otitis media and sinusitis involvements | 91 | |
| Sphenoidal sinusitis | 35 times | or 70.0% |
| Ethmoidal sinusitis | 22 times | or 44.0% |
| Frontal sinusitis | 17 times | or 34.0% |
| Otitis media | 17 times | or 34.0% |
| Pleurisy | 33 cases | or 37.9% |
| Empyema | 13 cases | or 15.0% |
| Meningitis | 7 cases | or 8.0% |
| Meningococcic | 4 cases | or 57.1% |
| Pneumococcic | 2 cases | or 28.6% |
| Tuberculous | 1 case | or 14.3% |
| Zenker's Degeneration | 7 cases | or 8.0% |
| Pulmonary tuberculosis | 4 cases | or 4.6% |
| Septicemia | 5 cases | or 5.8% |
| Lung abscess | 4 cases | or 4.6% |
| Pericarditis | 3 cases | or 3.4% |
| Typhoid fever | 3 cases | or 3.4% |
| Diphtheria | 3 cases | or 3.4% |
| Mediastinal abscess | 1 case | or 1.2% |
| Encephalitis, hemorrhagic | 1 case | or 1.2% |
| Abscess of kidney | 1 case | or 1.2% |
| Nephritis | 76 cases | or 21.3% |
| Mild | 34 cases | or 9.5% |
| Moderate | 40 cases | or 11.2% |
| Severe | 2 cases | or 0.6% |

| | |
|---|---|
| Peritonitis | 41 cases or 11.5% |
| Acute | 37 cases or 90.0% |
| Peritonitis from gunshot wound | 12 cases |
| Chronic | 4 cases or 10.0% |
| Gas Inhalation and Contact | 39 cases or 10.9% |
| Tracheobronchitis and pneumonia | 26 cases or 66.6% |
| Tracheobronchitis | 10 cases or 25.6% |
| Pneumonia | 3 cases or 7.8% |
| Tuberculosis, pulmonary | 36 cases or 10.1% |
| Chronic or healed | 24 cases or 66.7% |
| Acute | 8 cases or 22.2% |
| Chronic and acute | 4 cases or 11.1% |
| Septicemia | 32 cases or 9.0% |
| Streptococcus hemolyticus | 24 cases or 75.0% |
| Streptococcus, nonhemolyticus | 7 cases or 21.9% |
| Streptococcus viridans | 1 case or 3.1% |
| Meningitis | 22 cases or 6.1% |
| Meningococcic | 11 cases or 50.0% |
| Pneumococcic | 3 cases or 13.6% |
| Tuberculous | 1 case or 4.5% |
| Other types | 7 cases or 31.9% |
| Anemia from hemorrhage | 16 cases or 4.5% |
| Gas gangrene | 15 cases or 4.2% |
| Pericarditis | 14 cases or 3.9% |
| Acute | 9 cases or 64.3% |
| Chronic | 3 cases or 35.7% |
| Enterocolitis | 12 cases or 3.3% |
| Amebic | 3 cases or 25.0% |
| Bacillary | 2 cases or 16.7% |
| Undetermined | 7 cases or 58.3% |
| Zenker's degeneration | 9 cases or 2.5% |
| Typhoid fever | 8 cases or 2.2% |
| Diphtheria | 8 cases or 2.2% |
| Hemorrhagic encephalitis | 3 cases or 0.8% |
| Congenital anomalies | 14 cases or 3.9% |
| Kidney, horseshoe | 3 cases or 21.4% |
| Kidney hypoplasia | 2 cases or 14.3% |
| Kidney aplasia | 2 cases or 14.3% |
| Patent foramen ovale | 3 cases or 21.4% |
| Accessory lung | 1 case or 7.1% |
| Accessory pancreas | 1 case or 7.1% |
| Pectoral muscle, aplasia | 1 case or 7.1% |
| Meckel's diverticulum | 1 case or 7.1% |

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# TUMORS OF THE THYMUS IN MYASTHENIA GRAVIS

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# TUMORS OF THE THYMUS IN MYASTHENIA GRAVIS

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No characteristic lesions were described in the first seventeen autopsy reports of cases of myasthenia gravis. However, in 1901, Weigert reported a case with a thymic tumor and numerous foci of lymphocytes in the skeletal muscles. In the majority of autopsies since 1901, lymphocytic foci have been noted in the muscles, and the thymus has been frequently described as enlarged or the seat of a tumor. In this paper only the thymic abnormalities are to be considered.

Starr (1), in 1912, reviewed two hundred and fifty cases of myasthenia gravis, and noted that a pathologic condition of the thymus was recorded in twenty-eight per cent. of the cases that came to autopsy. But Starr evidently included all the earlier reports in which it is probable that the thymus was not carefully examined. Thus in Campbell and Bramwell's (2) review of seventeen autopsies in 1900 the thymus was not mentioned, although in recent autopsies it is very commonly described as abnormal. The percentage of cases with thymic involvement can therefore be more accurately determined by considering only the reports which have appeared since the publication of Weigert's paper.

Fifty-six autopsies have been published since 1901. The thymus was described as enlarged in seventeen of these cases; and in ten others it contained a tumor. Therefore some form of thymic involvement seems to occur in nearly half the cases of myasthenia gravis.

*Persistent or Enlarged Thymus.*—Enlargement or persistence of the thymus has been recorded in seventeen cases. These are listed in Table I, with the information as to actual size furnished by the respective authors. The data are not satisfactory in every case. Such divergent views are held as regards the normal weight of the thymus at different ages that one is not convinced of a true enlargement merely by the author's statement unsupported by any weights or measurements.

TABLE I

ENLARGEMENT OF THYMUS

| Author | Patient | Size of Thymus |
|---|---|---|
| Steinert,[3] | Elderly woman, | Persistent. |
| Meyer,[4] | Male, 47 years, | Enlarged. |
| Moorhead,[5] | Female, 25 years, | Persistent; extends over pericardium. |
| Booth,[6] | Male, 11 years, | 11 cm. × 5.5 cm. × 1 cm. |
| Marinesco,[7] | Female, 31 years, | Persistent. |
| Burr and McCarthy,[8] | Female, 21 years, | Enlarged. |
| Burr,[9] | Male, 30 years, | 22 gm. (abscess). |
| Hödlemoser,[10] | Female, 18 years, | Status thymico-lymphaticus, large thymus. |
| Symes,[11] | Female, 21 years, | Each lobe, 11.4 cm. × 3.8 cm. |
| Hart,[12] | Female, 30 years, | No age involution. |
| Dupré et Pagniez,[13] | Female, 32 years, | 8 gm., persistent. |
| Link,[14] | Male, 43 years, | 3 cm. long. |
| Boudon,[15] | Female, 17 years, | 36 gm. |
| Buzzard,[16] | Male, 40 years, | 9.5 gm. |
| Buzzard,[16] | Male, 40 years, | 59.4 gm. (cystic). |
| Buzzard,[16] | Female, 28 years, | 41 gm. |
| Schumacher & Roth,[17] | Female, 19 years, | 49 gm. |

The enlarged or persistent thymus in myasthenia is usually described as showing a normal histologic structure or a simple hyperplasia. In Burr's case the thymus contained a large abscess. Schumacher and Roth's case had exophthalmic goitre as well as myasthenia. The patient's myasthenic symptoms disappeared after thymectomy.

The frequent occurrence of hyperplasia of the thymus in myasthenia is of special interest in connection with the study of the thymic tumors which occur in this disease. Ten cases of myasthenia gravis have been recorded in which a tumor of the thymus was found at autopsy. It will be more convenient to describe my own case before discussing the literature.

The case to be reported occurred in the service of Dr. W. A. Jones at the Minneapolis City Hospital. The patient, a male 58 years old, presented the typical clinical picture of myasthenia gravis. A full clinical history is given by Dr. Jones in a paper read at the Detroit Meeting, June, 1916, and soon to be published in the Journal of the American Medical Association.

At autopsy[1] a tumor was found in the thymic region just above the heart and immediately behind the sternum. It was embedded in the loose connective tissues and was not adherent to the sternum or any of the thoracic viscera. Microscopic examination of the adipose tissue lying around the superior part of the tumor revealed the usual senile thymic tissue, viz., very small thymic lobules containing Hassall corpuscles, and separated by large quantities of adipose tissue. The tumor is therefore intimately related to the thymus gland which otherwise shows the typical senile involution.

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[1] The autopsy was performed by me, and a report of the findings was submitted to Dr. Jones.

The tumor was easily separated from the adjacent connective tissue. It weighed sixty grams. It is 6 cm. long, 3.5 cm. deep antero-posteriorly, and the transverse measurements are 3.6 cm. above and 4.2 cm. below. It is completely enclosed in a strong fibrous capsule, about 0.5 mm. thick, which is not perforated at any point. The external surfaces show low nodular elevations (Fig. 1). The cut surface is of soft consistence, somewhat softer than the thymus of a child, and is studed with hemorrhagic areas (Fig. 2). The tissue is of light gray color except in the hemorrhagic areas. No lobules are seen.

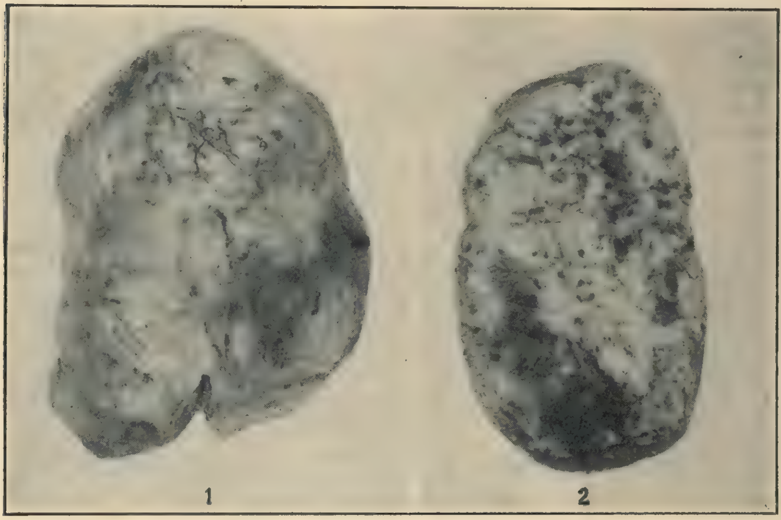

FIG. 1. Anterior surface of thymic tumor, after removal of the adjacent connective tissue. Photomicrograph. × 1.

FIG. 2. Cut surface of tumor. The dark areas are hemorrhagic. Photomicrograph. × 1.

Under low magnification no definite lobulation can be made out, although there are some irregular septa composed of very loose connective tissue, containing small lymphocytes and blood vessels. Numerous small hemorrhages are to be seen. Around these areas there are often many phagocytic cells filled with blood pigment. There are a few areas of necrosis.

The tumor tissue consists of cells with large vesicular nuclei and abundant light-staining cytoplasm, fused together to form a syncytium. Throughout the syncytium are numerous spaces of variable shape and size, containing small lymphocytes (Fig. 3, 4). In some areas the lymphoid cells predominate, in other areas the epithelial cells. To those familiar with the histogenesis of the thymus it will be clear that this tissue corresponds closely to the structure of the embryonic thymus at the stage when the epithelial organ is being transformed into a lymphoid organ. The cells with large clear nuclei are thymic epithelial cells. In another paper I (18) have published figures illustrating this stage in the development of the thymus. There is no true differentiation into cortex and medulla in any part of the tumor examined; but some areas contain many more lymphocytes than other portions (Fig. 5). It is to be remembered that the entire reticulum of the thymus is of epithelial origin, and that the cortex normally contains a great many more lymphocytes than the medulla—the latter retaining somewhat of an epithelial appearance in the fully formed gland.

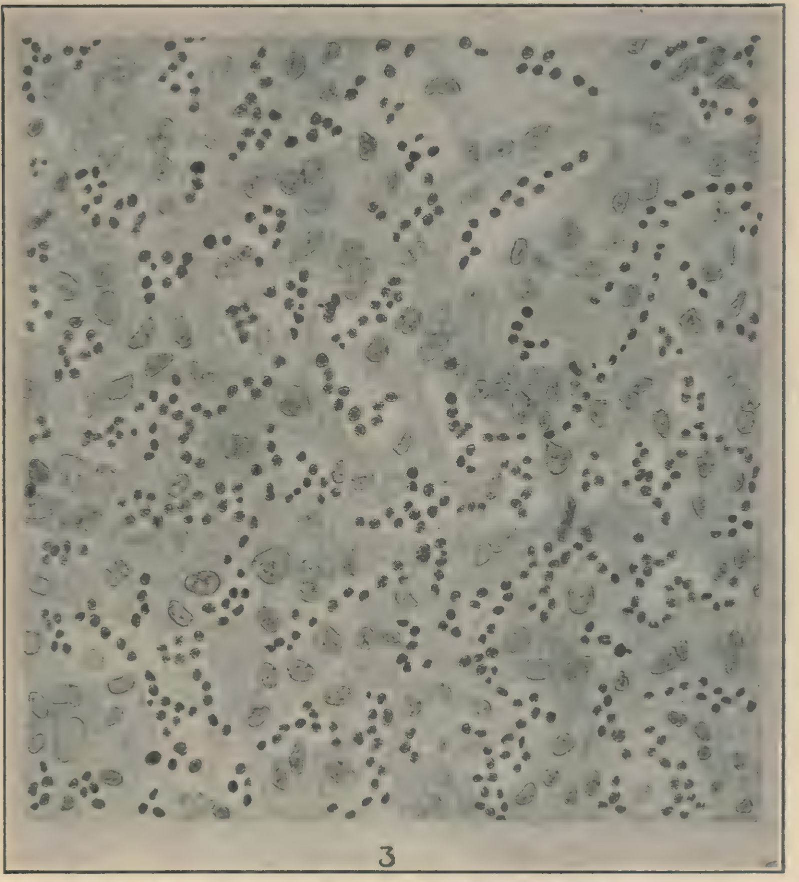

FIG. 3. A typical area of the tumor showing the epithelial reticulum and numerous lymphocytes in the spaces. This corresponds to the structure of the fetal thymus at the stage of the lymphoid transformation. Drawing. × 600.

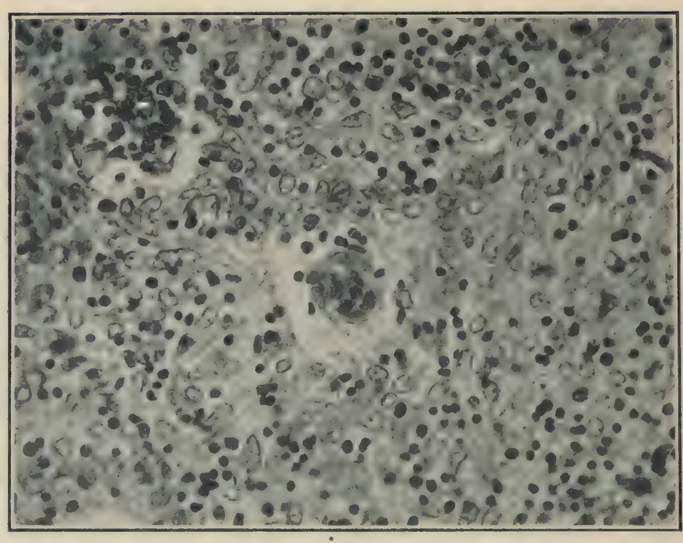

Fig. 4. Area of tumor. In the greater part of this area there are relatively few lymphocytes; the epithelium is therefore prominent. In the center of the field is a blood capillary surrounded by a large space containing only a few lymphocytes. Photomicrograph. × 500.

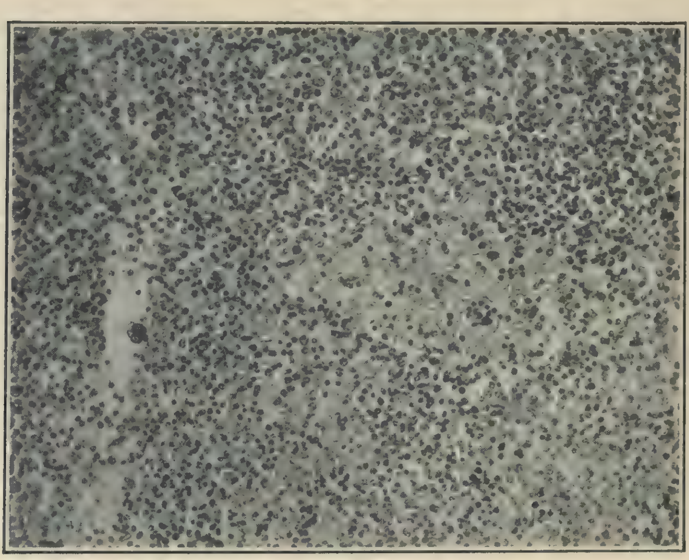

Fig. 5. Area showing a central portion composed almost entirely of epithelium, surrounded by tissue in which the lymphocytes are very numerous. This probably represents an early stage of differentiation into cortex and medulla. Photomicrograph. × 80.

No corpuscles of Hassall are to be seen. These structures do not appear in the development of the thymus until the medulla has become well defined, and since the tumor tissue has not yet attained this stage of differentiation one would not expect to find them. However, there can be no doubt that the tumor is composed of young thymic tissue, even though no corpuscles are present.

In some parts of the tumor there are tubule-like structures which contain one or more small blood vessels and a few lymphocytes (Figs. 4 and 6). The cavity is much larger than the contained vessel, and the epithelial syncytium is arranged in a smooth layer to form its wall. Examination of serial sections shows that these tubular cavities are branched off from the larger septa. Small blood vessels accompanied by lymphocytes penetrate the more solid parts of the tumor, forming cavities in the tissue much larger than the invading vessels. The chief interest of these structures is the danger of their misinterpretation. They might readily be mistaken for lymph vessels or tubules. Apparently Mandelbaum and Celler (22) did not recognize their true nature.

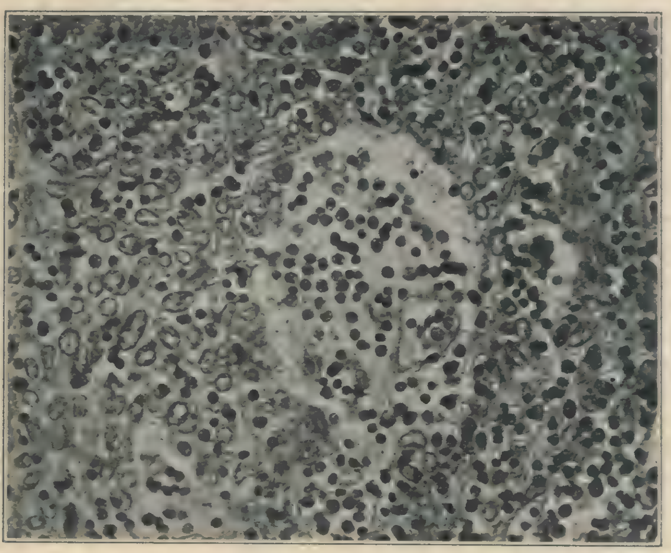

FIG. 6. In the center of the field is a large rounded space containing lymphocytes, in the upper right hand corner of which a blood capillary is shown. These spaces are continuous with the interlobular septa. They are not to be interpreted as tubules or lymph vessels. Photomicrograph. × 500.

This is evidently a benign tumor. It is completely encapsulated, and there are very few mitotic figures. It might properly be classified as an adenoma, but it is probably better in the present state of our knowledge of these growths to designate it simply as a benign thymoma.

Ten cases of thymic tumor associated with myasthenia gravis have been reported. These will be discussed separately.

1. Laquer and Weigert (19), 1901. Male, 30 years old. A reddish tumor, 5 cm. long, 5 cm. wide, and 3 cm. in thickness, was found in the thymic position in the anterior mediastinum close to the pericardium. The cut surface was partly dark, partly bright red. Microscopically, there were abundant free masses of blood surrounding islands of tissue. The tissue islands consisted mainly of lymphoid cells, such as are found in the normal thymus, and less numerous larger cells with abundant cytoplasm and large clear nuclei—the so-called epithelioid cells. Hassall's corpuscles were found. Tumor cells were found in the veins and arteries of the growth.

The lymphorrhages (foci of lymphocytes) in the muscles were interpreted by Weigert as metastases of the thymic growth; but these have been shown to occur in myasthenia in the absence of any thymic abnormality and can in no sense be regarded as metastases. This error is apparently chiefly responsible for Weigert's interpretation of his tumor as a lymphosarcoma. The presence of cells in the blood vessels (if these were lymphocytes) does not mean malignancy, since there is always a considerable increase of lymphocytes in the thymic blood vessels during the lymphoid transformation of the normally developing thymus. Weigert's description shows clearly that he saw the epithelial reticulum and the lymphocytes. He described fetal thymic tissue. His tumor is clearly a benign growth very similar to my case.

2. Weigert (19) mentions a mediastinal tumor, similar to the one first described, seen by him in an uncertain case of myasthenia. No further details are given. The tumor was apparently not examined microscopically.

3. Oppenheim (20), 1911. Female, 40 years old. A somewhat atypical case of myasthenia. A tumor the size of a five-mark piece was grown to the right lung. In the region of the thymus was a light brownish-red tumor the size of a mandarin. The cut surface was sprinkled with numerous red and black points and stripes. In stained preparations there were closely crowded masses of deeply stained round cells and large pale nuclei. Oppenheim's diagnosis was lymphosarcoma of the thymus. However, the size and gross appearance of the tumor correspond to Weigert's case. The microscopic description was not detailed, but apparently a reticulum and lymphocytes were present. It is probable that this was a benign tumor similar to Weigert's.

4. Hun, Blumer, and Streeter (21), 1904. Male, 32 years old. In the anterior mediastinal space behind the manubrium was a rather soft, almost fluctuating globular tumor, about 5 cm. in diameter, and

not connected with either trachea or esophagus. It was possibly a trifle lower than the usual position of the thymus. On section it was exceedingly soft and some parts were distinctly hemorrhagic. The nonhemorrhagic parts had a grayish-white color and the appearance of a cellular sarcoma. A connective tissue capsule surrounded the tumor. Microscopically it consisted of irregular columns or islands of cuboidal epithelioid cells with large granular protoplasmic bodies and rather large pale nuclei. Surrounding these were an almost equal number of small cells resembling the lymphoid cells of the thymus. The authors stated that the appearances suggested an hyperplasia of the thymus, but they concluded finally that the tumor was a lymphosarcoma. This error, as in Weigert's case, seems to have been due to their interpretation of the lymphorrhages in the muscles as metastases of the thymic tumor. The tumor corresponds closely both grossly and microscopically with my own case.

5. Mandlebaum and Celler (22), 1908. Male, 52 years old. Thymus weighed 20 gm.; it was 5 cm. in length, 3.5 cm. in width, and 2 cm. in thickness. The cut surface was pinkish in color, very firm and lobulated. In only a few places could any normal thymic tissue be found. This, in some instances, was separated from the tumor by layers of connective tissue, but in other places it was seen to be in direct relationship with the tumor. The tumor itself was separated from the surrounding structures by a well marked connective tissue capsule which extended into the tumor, dividing it into lobes. Scattered areas of blood pigment were seen in the connective tissue septa.

Microscopically, the tumor contained numerous widely dilated lymph channels or lymph spaces lined by endothelium. The tumor tissue between the lymph channels was of a very dense cellular structure. The cells were large, of an oval, polygonal, or irregular shape and contained large vesicular nuclei. The cytoplasm was granular and stained lightly with eosin or picric acid. Throughout the section the cells had a tendency to form fairly large concentric bodies, somewhat resembling those found in psammoma and certain types of endothelioma, but having no direct connection with any blood vessels. None of the cells showed any mitotic changes. In some of the sections small islands of lymphoid cells were seen scattered between the tumor cells. The lymph spaces were well filled with large spherical cells, smaller lymphocytic cells, and granular matter.

The authors believed that the tumor cells were all derived originally from the endothelium of the perivascular lymph spaces, and therefore concluded that the tumor should be classed as a perilymphatic lymphangio-endothelioma. Apparently no true Hassall corpuscles were found in the tumor proper.

It is probable, however, that the lymph channels described by Mandlebaum and Celler are only spaces in the epithelial tissue hollowed out by invading blood vessels, such as were described in my case (Figs. 4, 6). These spaces are lined not by a true endothelium but by a condensation of the adjacent epithelial cells (cf. their Fig. 7). A simpler explanation of this tumor is to regard the large cells as thymic epithelium, not as endothelium. With this interpretation the growth would differ from my case only in containing a much smaller proportion of lymphocytes. It would be classified as a benign thymoma composed largely of epithelial cells.

6. Wiener (23), in 1908, demonstrated a case of myasthenia before the Verein deutscher Aertze in Prague. The patient, a woman 67 years old, died later and at autopsy a tumor was found in the position of the thymus. No further details are given in this reference.

7. Meggendorfer (24), 1908. A case of myasthenia in a male 47 years old, regarded by the author as somewhat atypical. The veins of the right side of the thorax and neck were very prominent, apparently because of the thymic enlargement. The leucocyte count was 9,200, of which 39.5 per cent. were polymorphonuclears and 60 per cent. lymphocytes. Both lobes of the thyroid were enlarged. There was some albumen and at times sugar in the urine. No myasthenic reaction was present. At autopsy a firm nodular tumor mass was found in the position of the thymus. The aortic arch and its primary branches, the vena cava, and the left vagus nerve were surrounded by tumor tissue. On both sides the tumor was firmly adherent to the upper anterior parts of the lungs. Metastases were found in the visceral pleura of the right lung and in the diaphragmatic pleura of the right side. The cut surface was mainly firm with a few soft areas. Microscopically the tumor was described as a large round cell sarcoma with strongly developed fibrous connective tissue. No Hassall's corpuscles were found, but the author noted an unmistakable resemblance to thymic tissue.

It is unfortunate that the microscopic description of this case is so incomplete. It is not clear in what respect the tumor tissue resembles thymic tissue or how it differs histologically from the benign thymic tumors. There is a possibility that the tumor was primary in the lymph nodes, though this is improbable. This is the only case in the literature in which myasthenia was associated with a malignant thymic tumor. It is also the only case of myasthenia in which the thymic tumor was large enough to cause pressure symptoms.

8. Klose (25), 1912. Male, 23 years old. A sarcoma the size of a hen's egg was found in the thymus. Metastases were found in the muscles of the extremities and the diaphragm. (These were evidently only the lymphorrhages which are usually found in the muscles in myasthenia, independently of thymic tumors.) The thymic tumor is described as probably extirpable. Small Hassall corpuscles were found in the tumor. The description given of the tumor is so incomplete that no exact diagnosis can be made. It is evidently, however, not a sarcoma, since the author mistook the lymphorrhages for metastases. Judging from its size and shape it is presumably the same type of tumor found in my case.

9. Claude, Géry, and Porak (26), 1914. Male, 51 years old. An encapsulated tumor the size of a duck's egg was found in the position of the thymus. The external surface was nodular and a cyst the size of a hazelnut was found at the lower pole. On the cut surface the tumor was divided into lobules by connective tissue septa. Microscopically, there were numerous tubular structures lined by epithelium or endothelium. The cellular tissue between these structures in some places was arranged to form concentric bodies suggesting Hassall's corpuscles. The tissue was interpreted as epithelium with a sinusoidal circulation. The authors designated the tumor as an epithelioma. No lymphoid cells are mentioned.

This is presumably a benign thymoma composed almost entirely of epithelium. The significance of the tubular structures is not clear.

10. Hart (12). An acute case of myasthenia in a recruit (age not given). The thymic tumor measured 5 cm. in length, 4 cm. in width, and 4 cm. in thickness. Externally it was nodular and firm. Dense fibrous septa penetrated the tumor, appearing as an irregular network on the cut surface. Between the septa the tissue was soft and grayish-red in color; in some parts hemorrhagic areas were present. Some atrophic thymic tissue was found around the upper pole of the tumor. In the tumor lymphocytes were intermingled with large epithelioid cells, the latter predominating. Hart regarded his case as a special form of hyperplasia of the thymus, and not as a true neoplasm. This tumor is very similar to my case.

Of the eleven thymic tumors found in myasthenics at autopsy, five correspond very closely in gross and microscopic structure, viz., the cases of Laquer and Weigert, Oppenheim, Hun, Hart, and my own case. The tumors reported by Mandlebaum and Celler and by

Claude may be interpreted as benign tumors composed mainly of thymic epithelium and differing from the five cases just mentioned in that the lymphoid cells have infiltrated or developed only to a slight extent.

The earlier thymic tumor mentioned by Weigert, Wiener's case, and Klose's case are all too meagerly described for accurate classification, but the sizes and shapes of these tumors suggest that they belong to this group of benign epithelial growths of the thymus.

The thymic tumors occurring in myasthenia form a distinct group. They are all comparatively small benign growths, composed of young thymic tissue. Many are hemorrhagic. This type of tumor seems to occur only in myasthenia.

Meggendorfer's tumor is an exceptional case. It is the only malignant thymic tumor ever found in association with myasthenia; although a great many malignant thymic tumors of this type have been reported independent of this disease. It may be a coincidence such as Goldflam's (27) case of myasthenia with a malignant tumor of the lungs. Over sixty malignant tumors of the thymus have been reported and only one of these occurred in connection with myasthenia. It is therefore difficult to believe that the two conditions have any causal connection.

*Tumors of the Thymus Not Associated with Myasthenia.*—A large number of thymic tumors are recorded in the literature. Hoffmann (28), in 1896, collected thirty-five cases; and Rubaschow (29), 1911, added thirty-six more. It has been pointed out by Hoffmann and others that it is frequently impossible to determine the exact origin of a mediastinal tumor, and that many tumors are attributed to the thymus that may or may not have originated there. Statistics on thymic tumors are therefore to be used with some reservations. I have studied the reports of seventy-three thymic tumors, not including those occurring in myasthenia. Thymic enlargements in leukemia are not considered here because these are presumably merely infiltrations and not true neoplasms.

The seventy-three cases were grouped by the several authors as follows: 27 lymphosarcoma, 3 round cell sarcoma, 2 fibrosarcoma, 19 other varieties of sarcoma or sarcoma without special designations, 10 carcinoma, 6 thymoma, 1 fibroma, 5 miscellaneous. Carcinoma and sarcoma are not sharply distinguished. The small cell carcinoma of Rubaschow and Eisenstädt (30) would probably be classified as sarcoma by the majority of authors. Thymoma is apparently used synonymously with lymphosarcoma.

The prevailing type of malignant thymic tumor, usually called a sarcoma, consists of large cells of round or irregular shape, sometimes arranged in masses suggesting epithelium. A stroma is often mentioned but seldom carefully described. It does not seem justifiable to assume that tumor tissues of this type are of connective tissue origin. We know that malignant neuroblastomata often assume this appearance, and it is not uncommon to find the rapidly growing parts of malignant epithelial tumors of the kidney composed of loosely arranged rounded cells. It is easily possible that tumors arising from the thymic epithelium may take on this "sarcomatous" appearance when growing rapidly. Ewing (31) is of the opinion that thymic sarcomata develop from the reticulum, and his three cases make a strong argument in support of this interpretation. Ewing's Case III is especially valuable in showing a transition between the benign tumors, such as occur in myasthenia, and the very malignant tumors. The epithelial reticulum is very prominent and compact in the benign tumors; but as the rate of growth increases it becomes more and more drawn out and large cells separate from it until the free cells become more prominent than the reticulum. Lymphocytes seem to be less numerous in the more malignant growths.

The type cell in these tumors is probably a thymic epithelial cell. The term sarcoma will lose all its original significance if applied to tumors of this character, and it seems equally inappropriate to call them carcinoma. The term thymoma, suggested by Grandhomme (32), and employed by Thiroloix et Debré (33), Simmonds (34), and Ewing seems most suitable. A thymoma may now be defined as a tumor, benign or malignant, probably derived from the thymic epithelium, and usually identifiable by the epithelial reticulum and lymphocytes. Hassall's corpuscles are sometimes present. Thymoma as thus defined would include practically all the tumors classed as lymphosarcomata by various authors, the benign tumors occurring in myasthenia, and some cases described as small cell carcinomata. It would not include tumors in which the type cell is evidently not from the thymic epithelium, such as Lange's (35) fibroma and Schneider's (36) fibrosarcoma.

It was mentioned above that Hart does not regard the thymic growths found in myasthenia as true tumors, but as a special form of hyperplasia of the organ. The fact that there is a uniform hyperplasia of the entire thymus in many cases of myasthenia gives support to Hart's interpretation. It is indeed probable that the thymic tumors and the diffuse thymic hyperplasias in myasthenia are due to the same underlying cause; but, since we do not know the cause either of myasthenia or true tumors, there can hardly be any objection to classifying a well-defined encapsulated growth, such as I have described, as a true neoplasm.

Lymphorrhages have been found in the muscles and internal organs in a large per cent. of recent autopsies. No satisfactory interpretation of these lesions has been given. Their occurrence is not dependent upon the condition of the thymus. They were very numerous in the liver in my case, and a few were found in the adrenal. Several pieces of the neck muscles were examined but no lymphorrhages were found. Unfortunately, other muscles were not preserved.

Thymic lesions cannot be regarded as the cause of myasthenia, since they are present in only about half the cases. Probably the abnormal thymus is due to some more fundamental disorder which is also responsible for the muscle weakness and other features of the disease. The hyperplastic thymus of Graves' disease is usually interpreted in this way.

## Summary

A benign thymic tumor from a typical case of myasthenia gravis is described.

The tumor is composed of thymic tissue of a fetal type, *i. e.,* a dense epithelial reticulum with lymphocytes. The usual thymic rests were found in the adipose tissue around the growth.

The thymic tumors occurring in myasthenia gravis seem to form a distinct group unlike any other thymic tumors. They may be classified as benign thymoma.

Some abnormality of the thymus is found in nearly half the cases of myasthenia gravis.

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# Effect of Foreign Protein on the Kidney

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# EFFECT OF FOREIGN PROTEIN ON THE KIDNEY

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It is known that some persons develop symptoms of anaphylactic character after eating certain foods, of which shellfish, egg-white, and certain fruits are familiar examples. It is believed by many that asthma, urticaria and hay-fever have their origin largely in a condition of hypersensitiveness to special foreign proteins. The symptoms produced when a foreign protein is introduced into a sensitized animal are usually referred to a hypothetical substance—"anaphylatoxin"—formed by the action of antibody on antigen. There are reports in the literature to the effect that anatomic changes in the tissues occur in acute anaphylaxis, and Longcope[1] has advanced the theory that repeated anaphylactic shock may be responsible for such chronic diseases as nephritis. By a series of animal experiments Longcope believes that he has shown that repeated nonfatal poisoning with foreign protein is responsible for some cases of chronic nephritis, cirrhosis of the liver, and chronic myocarditis. Our interest in the etiology of chronic nephritis led us to repeat some of his experiments.

## ANATOMIC CHANGES IN ACUTE ANAPHYLAXIS

Gay and Southard[2] described hemorrhages and areas of fatty degeneration in guinea-pigs that died in acute anaphylactic shock and in those killed subsequent to severe shock. The hemorrhages were widely distributed, but were most commonly found in the mucosa of the stomach and in the lungs. Areas of fatty degeneration were found in capillary endothelium, in the mucosa of the stomach, and in the cardiac and voluntary muscle. The hemorrhages were interpreted as the result of the degeneration of the capillary endothelium. But Anderson and Rosenau[3] in repeating this work could find no evidence

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[1] Jour. Exper. Med., 1913, 18, p. 678; 1915, 22, p. 793; Trans. Assn. Am. Phys., 1914, 29, p. 49.

[2] Jour. Med. Research, 1907, 16, p. 143; 1908, 18, p. 17.

[3] Jour. Med. Research, 1908, 19, p. 57.

of fatty degeneration. They did not find the hemorrhages so constantly and seem inclined to interpret them, when present, as due to the violent struggling of the animal during shock rather than to the action of the protein.

It is known also that fatty degeneration does not occur suddenly. Usually several days are required for a cell to become definitely fatty, and very little fat accumulates earlier than 48 hours after the injury. It is inconceivable that this change could occur within a few minutes or even within a few hours. Again the normal variations in the fat content of voluntary muscle fibers are so great that one can seldom speak with assurance of a fatty degeneration in this tissue. These considerations lend strong support to the negative findings of Anderson and Rosenau.

The Arthus[4] phenomenon seems to lend experimental support to the view that foreign protein may produce local anatomic changes when introduced into a sensitive animal.

Longcope[5] studied the effects of repeated injections of foreign protein in guinea-pigs, rabbits, dogs and cats. Small sensitizing doses of horse serum or egg-white were given 20-25 days before the secondary injections were begun. The secondary injections were spaced and regulated with the object of producing shock a number of times. Some of the experiments lasted as long as 3 months, during which time as many as 15 injections were given. Not all injections resulted in anaphylactic symptoms and many of the reactions were recorded as slight.

Changes were found in the kidneys in 23 of 29 rabbits, and in a large proportion of the other animals experimented on. In the animals that died acutely diffuse degenerative changes were found. In those that lived longer, extensive round cell infiltration was the most striking change. Dense fibrous rays with atrophic tubules were found in 6 rabbits. Six rabbits showed normal kidneys at necropsy. Portal cirrhosis of the liver and areas of chronic myocarditis were also found frequently.

In a more recent paper Longcope[6] found that similar though less marked renal lesions may be produced by a single large injection of foreign protein, or by several injections in which a different protein was used each time. All the sensitized animals developed nephritis, but of those that received a single large injection, or several different proteins, only one-third developed nephritis. He concluded that foreign protein is itself toxic and may produce nephritis, but that previous sensitization increases the extent, severity and constancy of the lesions.

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[4] Arch. internat. de Physiol., 1908-9, 7, p. 471.

[5] Jour. Exper. Med., 1913, 18, p. 678.

[6] Ibid., 1915, 22, p. 793.

The lesions described by Longcope correspond in all respects with those of ordinary spontaneous nephritis. As controls, 24 supposedly normal rabbits were studied, and in 4 of these a few small areas of round cell infiltration were found similar to those occurring in the experimental animals. The argument that the lesions were produced by the foreign protein because they were more extensive and severe than in the controls has no value, since advanced stages of spontaneous nephritis are of common occurrence. The controls apparently did not have the same opportunity to contract an infection, since there is no mention of their having been kept under the same conditions and for the same length of time as the experimental animals. It is also to be noted that some of the animals may have had nephritis at the outset of the experiment, since it is admitted that animals with a faint trace of albumin were used. Again, there was no control at all for the healed stages of nephritis, of which 6 cases are mentioned and attributed to the action of the protein, since these do not cause albuminuria. No cultures were taken from the kidneys to determine whether the nephritis was due to infective or to chemical irritants.

Three of Longcope's experiments were controlled somewhat better. Three dogs were operated on, and a portion of one kidney from each was removed for examination before the secondary inoculations were begun. The excised portions of the kidneys were found normal, but each animal developed nephritis after a number of injections of protein subsequent to the operation. But no cultures were made from these kidneys, and a renal infection was therefore not excluded.

The uncertainty of the results of Longcope's work is indicated further by his attributing the ordinary cirrhosis of the liver of rabbits to the action of the protein. This is an unusually common lesion. It occurs in fully three-fourths of adult rabbits and is probably due to bacterial infection. All who have studied rabbit livers extensively recognize this as a spontaneous lesion.

Longcope's conclusions were confirmed by Boughton[7] to the extent that repeated injections of foreign protein may produce renal lesions.

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[7] Jour. Immunology, 1916, 1, p. 105.

Boughton produced repeated anaphylactic shock in guinea-pigs, using beef serum and egg-white as antigens. Some of the animals received as many as 19 secondary injections, and the average number of injections was 9. The average duration of the experiments was 4 months, but some animals were under experimentation as long as 8 months. The urine was not examined systematically. Twenty-three guinea-pigs were used. Degeneration, desquamation, and necrosis of the tubular epithelium was the most striking change noted and was seen in all cases. Round cell infiltration was found in nearly all cases, though it was never vary marked. There was no distinct scar formation.

Six controls were given injections of protein so spaced as to avoid the production of anaphylaxis. Tubular injury was much less marked than in the sensitized animals. Areas of round cell infiltration were also seen, but these were only one-tenth to one-fifteenth as frequent as in the sensitized animals.

All injections were given intraperitoneally and ranged as high as 10 c c (undiluted serum, or 50% solution of egg-white). The kidney lesions were regarded as subacute rather than chronic.

Boughton seems to conclude that foreign protein produces some renal injury when injected without the production of anaphylactic shock, and rather severe injury when this phenomenon is repeatedly produced. The tubular injury was the most prominent lesion, and the production of a typical chronic nephritis is apparently not claimed. As regards the interpretation of the round cell infiltration, the sources of error are the same as in Longcope's experiments.

## EXPERIMENTS

The purpose of our investigation was to determine whether chronic nephritis can be experimentally produced in rabbits by a foreign protein. Repeated shock was produced in a few animals, but in most of the experiments no attempt was made to produce anaphylaxis.

Special effort has been made to eliminate various sources of error. All rabbits that had albuminuria were discarded. In order to eliminate the occasional inactive cases of spontaneous nephritis, rabbits with normal urine were operated on with aseptic precautions under ether anesthesia in order to expose and examine the surface of the left kidney. All except early cases of nephritis may be recognized by the presence of spots and pits on the surface of the kidney. Both kidneys are nearly always involved to about the same extent, so that it is not necessary to examine the right kidney. About one week after the operation, the experiment was begun in case the wound was completely healed and the urine free from albumin. Ascitic fluid, egg-albumin, and sheep serum were used. Injections were made intravenously except as otherwise indicated.

### ASCITIC FLUID WITH ANAPHYLAXIS

*Rabbit 25.*—Weight 2,000 gm. July 3: Test for albumin negative. Left kidney exposed and found normal.

July 12, 17, 24: 1 c c sensitizing injections of ascitic fluid.
Sept. 10: 1 c c, moderate anaphylactic symptoms.
Sept. 21: 0.5 c c, slight symptoms.
Oct. 5: 0.25 c c, slight symptoms.
Oct. 15: 0.5 c c, slight symptoms.
Oct. 25: 0.5 c c, moderate symptoms.
Nov. 5: 0.5 c c, moderate symptoms.
Nov. 15: 1 c c, severe symptoms.
Nov. 21: Found dead.

Tests for albumin negative, Sept. 10, 25, Oct. 11. Trace of albumin, Nov. 7. Seven secondary injections were given over a period of 71 days. At necropsy the kidneys showed no gross changes. Microscopically an occasional small focus of lymphocytes was found deep in the cortex.

*Rabbit 35.*—Weight 1,885 gm. Sept. 11: Test for albumin negative. Left kidney exposed and found normal.

Oct. 5: 0.25 c c, ascitic fluid.
Oct. 15: 0.5 c c, slight symptoms.
Oct. 25: 0.5 c c, moderate symptoms.
Nov. 5: 1 c c, moderate symptoms.
Nov. 15: 1 c c, slight symptoms.
Nov. 26: 2 c c, slight symptoms.
Dec. 6: 2.5 c c, moderate symptoms.
Dec. 17: 2.5 c c, slight symptoms.
Dec. 27: 2.5 c c, slight symptoms.
Jan. 10: Killed.

Tests for albumin negative, Nov. 8; positive, Jan. 4 and 10. On Dec. 5, the serum from this rabbit gave a strong precipitin test with human blood serum. Eight secondary injections were given over a period of 87 days. At necropsy the kidneys showed no gross changes. Microscopically an occasional small lymphocytic focus was found in the cortex.

*Rabbit 33.*—Weight 2,320 gm. July 18: Test for albumin negative. Left kidney exposed and found normal.

Sept. 10: 0.5 c c, ascitic fluid.
Sept. 21: 0.5 c c, severe symptoms.
Oct. 5: 0.25 c c, slight symptoms.
Oct. 15: 0.25 c c, slight symptoms.
Oct. 25: 0.5 c c, moderate symptoms.
Nov. 5: 0.5 c c, moderate symptoms.

Nov. 15: 1 c c, slight symptoms.
Nov. 26: 2 c c, slight symptoms.
Dec. 6: 2.5 c c, slight symptoms.
Dec. 17: Found dead.

Tests for albumin negative, Sept. 10, 25, Oct. 11, Nov. 7. On Dec. 4 the serum from this rabbit gave no precipitate with human blood serum. Eight secondary injections were given over a period of 87 days. The kidneys showed no lesions grossly or microscopically.

### Ascitic Fluid Without Anaphylaxis

*Rabbit 32.*—Weight 2,320 gm. Oct. 2: Test for albumin negative.

Oct. 6: Left kidney exposed and found normal.

One c c ascitic fluid, Oct. 16, 23.

Two c c, Oct. 27, Nov. 1, 6, 12, 17.

Three c c, Nov. 22.

3.5 c c, Nov. 27, Dec. 3, 8, 13, 18, 22, 26, 31.

Tests for albumin negative, Oct. 13, Nov. 9, 22, Dec. 13; positive, Jan. 3.

Killed, Jan. 3. 16 injections were given over a period of 79 days.

No gross changes found in the kidneys. Microscopically a few small lymphocytic foci found in the cortex.

*Rabbit 54.*—Weight 2,105 gm. Jan. 28: Test for albumin negative.

Jan. 31: Left kidney exposed and found normal. Injections of ascitic fluid as follows: Feb. 11, 0.5 c c; Feb. 19, 0.75 c c; Feb. 23, 1 c c; Feb. 28, 0.75 c c; Mar. 5, 0.75 c c; Mar. 12, 1 c c; Mar. 19, 1 c c; Mar. 26, 1 c c; Apr. 3, 1 c c.

Killed, Apr. 8.

Albumin tests negative Feb. 14, 21, Mar. 2, 26, Apr. 5. Nine injections were given over a period of 56 days. No gross or microscopic lesions found in the kidneys.

*Rabbit 65.*—Weight 2,170 gm. Dec. 6: Albumin test negative. Left kidney exposed and found normal. Injections of ascitic fluid as follows: Dec. 22, 0.25 c c; Dec. 26, 0.5 c c; Dec. 31, 0.5 c c; Jan. 11, 0.75 c c; Jan. 22, 1 c c; Feb. 5, 1.5 c c (slight shock); Feb. 11, 1.5 c c; Feb. 19, 1.5 c c; Feb. 23, 2 c c; Feb. 28, 1 c c; Mar. 5, 1 c c; Mar. 12, 1 c c.

Killed Mar. 18. Twelve injections were given over a period of 86 days. Albumin tests negative, Dec. 14, Jan. 3, 30, Mar. 2. No gross or microscopic lesions found in the kidneys.

### Experiments with Egg-Albumin

*Rabbit 44.*—Weight 2,250 gm. Jan. 3: Test for albumin negative.

Jan. 5: Left kidney exposed and found normal.

Injections of a 1% solution of egg-albumin as follows: Jan. 15, 0.25 c c; Jan. 22, 0.5 c c; Jan. 26, 0.75 c c; Jan. 30, 1 c c; Feb. 5, 1.5 c c; Feb. 11, 2 c c of a 50% solution of egg-white intraperitoneally. Death, Feb. 11.

Albumin tests negative, Jan. 15, 25, 30. At necropsy the kidneys were found slightly swollen and moist. There was precipitated albumin in the capsular spaces and in the lumina of the convoluted tubules. Whether this albumin was excreted egg-albumin or serum-albumin was not determined. There were no areas of lymphocytic infiltration.

*Rabbit 50.*—Weight 1,850 gm. Feb. 28: Albumin test negative. Left kidney exposed and found normal. Intraperitoneal injections of a 50% solution of egg-albumin as follows: Mar. 14, 0.5 c c; Mar. 19, 1 c c; Mar. 26, 1 c c; Apr. 3, 1 c c; Apr. 8, 1 c c; Apr. 11, 1 c c; Apr. 16, 1 c c. Death, Apr. 19.

Albumin tests negative, Mar. 19, Apr. 5, 19. Seven injections were given during a period of 36 days. No gross or microscopic lesions found in the kidneys.

*Rabbit* 60.—Weight 1,560 gm. Dec. 5: Test for albumin negative.

Dec. 6: Left kidney exposed and found normal.

One c c of a 1% solution of egg-albumin was injected intravenously on the following dates: Dec. 20, 26, 31, Jan. 11, 22, and Feb. 5. One c c of a 50% solution of egg-white was injected intraperitoneally on Feb. 11, 19, 23, 25, and Mar. 5. Death, Mar. 9.

Albumin tests negative Dec. 14, Jan. 5, 15, 30, and Feb. 25. Trace of albumin, Jan. 25.

No gross changes found in the kidneys. Microscopically an occasional cast was observed, but there were no areas of lymphocytic infiltration.

### EXPERIMENTS WITH SHEEP SERUM

*Rabbit 38.*—Weight 1,950 gm. Dec. 3: Albumin test negative. Dec. 6: Left kidney exposed and found normal. Injections of sheep serum, diluted with equal volume of salt solution, as follows: Jan. 19, 0.5 c c; Jan. 24, 0.75 c c; Jan. 29, 1 c c; Feb. 5, 1.5 c c. Found dead, Feb. 6.

Albumin tests negative, Jan. 28, Feb. 1. The kidneys were swollen and cloudy. Precipitated albumin was found in the lumens of some of the convoluted tubules. There were no areas of lymphocytic infiltration.

*Rabbit 42.*—Weight 1,750 gm. Jan. 4: Test for albumin negative. Jan. 15: Left kidney exposed and found normal. Jan. 19, 0.5 c c of sheep serum. Jan. 22, found dead. Kidneys were cloudy. No microscopic examination.

*Rabbit 53.*—Weight 2,970 gm. Feb. 7: Left kidney exposed and found normal. Feb. 14: Test for albumin negative. Injections of sheep serum as follows: Feb. 19, 0.5 c c; Feb. 23, 0.75 c c; Feb. 28, 0.5 c c; Mar. 5, 0.5 c c; Mar. 12, 0.5 c c; Mar. 21, 1 c c; Mar. 26, 1 c c; Apr. 3, 1 c c. Apr. 4, found dead.

Albumin tests negative, Mar. 12, 21. Kidneys showed no gross changes. Microscopically precipitated albumin was found in the lumina of many of the tubules. There were a few casts. No areas of lymphocytic infiltration seen.

*Rabbit 63.*—Weight 1,860 gm. Feb. 7: Test for albumin negative. Left kidney exposed and found normal. Injections of sheep serum as follows: Feb. 19, 0.5 c c; Feb. 23, 0.75 c c; Feb. 28, 0.5 c c; Mar. 5, 0.5 c c; Mar. 12, 0.5 c c; Mar. 21, 1 c c; Mar. 26, 1 c c; Apr. 3, 1 c c; Apr. 4, found dead.

Albumin tests negative, Feb. 14, Mar. 21. Trace of albumin, Mar. 12. Several small pits were found on the surfaces of each kidney. Microscopically there were small areas of lymphocytic infiltration corresponding to the surface pits.

*Rabbit 67.*—Weight 1,960 gm. Jan. 4: Test for albumin negative. Jan. 5: Left kidney exposed and found normal. Injections of sheep serum as follows: Jan. 19, 0.5 c c; Jan. 24, 0.75 c c; Feb. 5, 1 c c; Feb. 19, 1 c c; Feb. 23, 1.5 c c; Feb. 28, 1 c c; Mar. 5, 1 c c; Mar. 12, 1 c c; Mar. 21, 1 c c; Mar. 26, 1 c c; Apr. 3, 1 c c; Apr. 11, 1 c c; Apr. 16, 1 c c; Apr. 18, 0.5 c c. Killed, Apr. 30.

Albumin tests negative, Jan. 17, 28, Feb. 25, Mar. 14; positive, Apr. 4, 18. A number of fairly deep pits were seen on the surfaces of the kidneys. Microscopically rather extensive lymphocytic infiltrations were found underlying the pits. The lesions correspond with those of a well advanced spontaneous nephritis.

*Rabbit 113.*—Weight 1,824 gm. May 14: Albumin test negative. Left kidney removed and found normal grossly and microscopically. Injections of sheep serum as follows: May 28, 0.5 c c; June 4, 0.75 c c; June 13, 2 c c; June 27, 2 c c; June 29, 1 c c; July 2, 2 c c. Killed, July 11. Loss of weight during experiment, 474 gm. Albumin tests positive, June 27, July 2, 10. The right kidney was covered with pits. Rather large areas of lymphocytic infiltration were found deep in the cortex underlying the pits, the appearances being those of a spontaneous nephritis. Cultures from the kdney in dextrose broth gave a pure growth of a streptococcus.

*Rabbit 103.*—Weight 1,250 gm. May 2: No albumin. Left kidney exposed and found normal. Injections of sheep serum as follows: May 28, 0.5 c c; June 4, 0.75 c c; June 6, 0.75 c c; June 13, 2 c c; June 27, 2 c c; June 29, 1 c c; July 2, 2 c c. Killed, July 6. No loss of weight. Albumin tests positive, June 27, July 3. The kidneys showed many fairly deep pits. Microscopically there were rather large areas of lymphocytic infiltration. The appearances were those of a spontaneous nephritis. Cultures from the kidneys taken immediately after death gave a pure growth of a streptococcus.

## DISCUSSION

These experiments indicate that ascitic fluid does not injure the kidney of a rabbit when injected intravenously in moderate amounts. The occasional small foci of lymphocytes found at necropsy in the injected animals can hardly be attributed to the proteins, since such lesions are commonly found in nonexperimental animals and were not excluded by the preliminary examination. Lesions of this size produce no changes on the kidney surface and seldom cause albuminuria.

The production of anaphylactic symptoms repeatedly did not cause nephritis. The symptoms were usually rather mild, but it is not possible to produce severe shock repeatedly. The animals either die or become refractory. The symptoms were apparently nearly as pronounced as in the experiments of Longcope and Boughton.

Egg-albumin in large doses seemed to cause a mild parenchymal injury of the kidney occasionally, but no chronic changes of any kind were produced. This protein seems to be only mildly toxic for rabbits.

Sheep serum, however, is evidently toxic for rabbits, especially in relatively large doses. Several animals died shortly after a single injection. It produces a tubular injury to the kidney, but this is not severe enough to cause the death of the animal. There is no evidence of special glomerular injury. Degenerative changes only were observed in the first three experiments; but during the summer inflammatory renal lesions were found in 4 rabbits. This is evidently the same lesion which Longcope obtained. It undoubtedly developed during the course of the experiments, since it was not present at the beginning. It is similar in all respects to ordinary spontaneous nephritis. Like the spontaneous disease it would seem to be due to a bacterial infection, since pure cultures of streptococci were obtained from the kidneys of rabbits 113 and 103.

Our interpretation of these apparently positive results with sheep serum is, therefore, that the animals acquired an infection during the course of the experiments. This infection may have been contracted from other rabbits in the animal house, or it may have been introduced by the intravenous injections, as these were not made with complete aseptic precautions. In either case it is probable that the injury of the kidney by the sheep serum favored the development of the infection. The injured kidney may have been a locus minoris resistentiae.

If this interpretation be correct it may be said that injections of sheep serum cause nephritis in rabbits by injuring the kidney and giving opportunity for the lodgment and growth of bacteria which may gain access to the circulation; but this is not a nephritis due directly to the action of anaphylatoxin or foreign protein. It is conceivable that toxic foreign protein might have a similar effect in man, but it is unlikely that such proteins ever gain access to the circulation in such relatively large amounts as are used experimentally in animals. And finally, if we grant the possibility that toxic foreign protein may enter the circulation and injure the kidney, we would expect the development of lesions similar to those found in the rabbit. But the only human renal lesions comparable to spontaneous nephritis of the rabbit are the hematogenous infections, namely, abscess of the kidney, descending pyelonephritis, and acute interstitial nephritis. Aside from the arteriosclerotic kidney, nearly all cases of chronic nephritis in man are examples of chronic glomerulo nephritis which has no anatomic resemblance whatever to spontaneous nephritis in the rabbit. It is therefore clear that the experimental production of such a lesion as spontaneous nephritis does not throw any light on the etiology of chronic nephritis in man.

It is not improbable that the various experimenters, who believe they have produced a chronic nephritis experimentally with uranium, potassium chromate, foreign protein, streptococci, etc., have been dealing with a spontaneous nephritis which was contracted during the experiment and hastened in its development by renal irritants. But even if all these claims were true we still have made little progress in determining the etiology of chronic nephritis in man.

As regards the etiology of human chronic nephritis we share the view, now widely held, that streptococci are the main causative agents, and that they first produce acute glomerular lesions which in many instances heal, but in others pass into a chronic stage. The continuous escape of streptococci from a focal infection or through a diseased mucous membrane would theoretically favor the development of chronic glomerular lesions. But thus far no one has been able to furnish experimental proof to support this theory.

## SUMMARY

Nontoxic foreign proteins, such as ascitic fluid, do not produce renal injury of any kind in the rabbit when injected intravenously in moderate amounts. The repeated production of anaphylactic symptoms with this protein likewise does not injure the kidney.

Egg-albumin may produce slight parenchymal injury when injected in large amounts, but usually it causes no damage.

Toxic foreign proteins such as sheep serum produce considerable parenchymal injury to the kidney which in some instances seems to lead to the rapid development of a renal infection comparable in all anatomic respects to the ordinary type of spontaneous nephritis. The fact that streptococci were isolated in pure culture from the kidneys of such animals shows that the lesion is essentially infective, and not the direct result of foreign protein or anaphylatoxin.

No lesion comparable to human chronic glomerulo-nephritis has been produced experimentally.

There is no experimental evidence that foreign protein is in any way responsible for chronic nephritis in man.

# Spontaneous Nephritis in Rabbits and Its Relation to Chronic Nephritis in Man

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# SPONTANEOUS NEPHRITIS IN RABBITS AND ITS RELATION TO CHRONIC NEPHRITIS IN MAN

WITH ONE PLATE

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It is well known that spontaneous[1] nephritis is often found in rabbits, and there are frequent estimates in the literature as to its frequency. There is, however, not much detail published as to the finer structure and pathogenesis of this lesion, and it is still a serious source of error for those engaged in the study of experimental nephritis. It is noteworthy that no investigator who claims to have produced a chronic nephritis experimentally has been able to draw a sharp distinction between the spontaneous lesions and those supposedly produced by the experimental procedure. This fact is usually admitted, though sometimes ignored, and the argument is made that a lesion occurring much oftener in the experimental than in the control animals is due to the experimental procedure. It is not generally recognized that nephritis in animals (especially the rabbit) is entirely different in its pathogenesis from human chronic nephritis, and that therefore the successful experimental production of lesions of this character does not necessarily illuminate the problems of the etiology of human chronic nephritis. We have, therefore, thought it worth while to give a brief account of the etiology and pathogenesis of spontaneous nephritis and to call attention to its relations to the problems of human nephritis and experimental nephritis.

## FREQUENCY

Smith[2] found some renal lesion in 11 of 50 rabbits. Ophüls,[3] in a study of the kidneys of 50 rabbits, found 28 entirely normal, 9 with slight parenchymatous lesions, 3 with a few small areas of cellular infiltration, and 10 with "scattered small areas in which were marked interstitial lesions with formation of small depressions on the surface."

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[1] "Naturally acquired," suggested by MacNider, is a more accurate term than "spontaneous," but the latter seems to be in general use.

[2] Arch. Int. Med., 1911, 8, p. 468.

[3] Proc. Soc. Exper. Biol. and Med., 1911, 8, p. 75.

Ghoreyeb[4] mentions a spontaneous lesion in 9 rabbits in an article in which 48 rabbits are recorded by number. Longcope[5] found lesions in the kidneys of 8 of 24 supposedly normal rabbits. Major[6] found that of "52 rabbits dead of natural causes, none had the extensive scarring of the cortex" such as was found in his experimental animals. Apparently Major was writing only of severe lesions.

In our own experience, which includes the study of over 200 rabbits over a period of two years, the percentage with spontaneous nephritis varied greatly from month to month. Sometimes nearly all were found normal, but usually from 10-20% were diseased. There were two periods during the past two years, each lasting about 1 month, when over 80% of the rabbits examined had nephritis. The disease apparently assumes an endemic form at times. The animals used were purchased in large groups from different dealers, and it was noted that the disease was much more prevalent in some groups than in others when they were first put into the animal house. Many rabbits developed nephritis after they came into our possession.

In view of the great variations we have noted, it seems unsafe to conclude, as Longcope and others have done, that a given lesion is due to the experimental procedure merely because it occurs oftener in the experimental than in the control animals. The source of the animals used as controls and the length of time they are kept in the animal house are very important factors.

## ETIOLOGY

We have attempted to isolate bacteria in eight cases of spontaneous nephritis by removing pieces of the kidney aseptically immediately after the animal had been killed, or from the living animal under ether anesthesia. The pieces of tissue were put in plain broth or in dextrose broth. Signs of growth first appeared in the medium after several days' incubation. Four of the cases gave no growth. In two cases we obtained a gram-positive staphylococcus in pure culture. These organisms when first isolated were very virulent — ¼ c c of a broth culture being a fatal dose for a rabbit. The third subculture, however, was practically avirulent.

In two other cases pure cultures of streptococci were obtained. These grew feebly and did not cause death or infection when inoculated into rabbits. We did not succeed in reproducing nephritis by injecting normal rabbits with the organisms obtained from any of the diseased kidneys. In the absence of this evidence the organisms isolated cannot with certainty be regarded as the etiologic agents; but it seems to us a highly probable assumption. They can hardly be due to contamination since the growth did not appear until after several days' incubation, the organisms were in pure culture, and in two instances were very virulent. It is known to us that streptococci have often been obtained from the tissues of rabbits, but these have usually been from animals that died slowly from natural or experimental disease where the opportunity for agonal invasion was present, and not from animals killed and cultured directly. Dr. W. P. Larson of this laboratory tells us that he has repeatedly found the normal kidneys of rabbits sterile.

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[4] Jour. Exper. Med., 1913, 18, p. 29.

[5] Ibid., 1913, 18 p. 678.

[6] Jour. Med. Research, 1917, 35, p. 349.

## PATHOLOGICAL ANATOMY AND GENESIS

For the sake of clearness we shall discuss this topic without special reference to observations recorded in the literature. In the earliest stages small foci of lymphocytes are found usually near the corticomedullary junction but sometimes elsewhere in the cortex. There are only a few of these lymphocytic foci in mild cases — many sections of the kidney may be entirely normal. But in severe cases these areas are numerous, and they may fuse to form extensive areas of infiltration. The lymphocytes are of intermediate size and lie for the most part closely packed between the tubules, but a few invade the epithelial cells (Fig. 1). At this early stage there are no gross changes on the surfaces of the kidneys. In severe cases the cut surface is clouded. Albumin is present in the urine except in cases where the foci are few and small.

The pressure of the exudate soon causes atrophy and disintegration of the parts of the tubules involved in the lymphocytic foci, and the destruction of these tubules is responsible for the changes that occur subsequently. The first gross changes noted are the appearance of a reddish or purplish spot on the surface of the kidney overlying the lymphocytic focus. Later on this purplish spot gradually sinks and a distinct funnel-shaped depression develops in its place. In the final stages the pit may be as deep as the thickness of the cortex. When the surfaces are studded with such deep pits the kidney assumes a shrunken scarred appearance (Fig. 2).

Microscopic sections through the purplish surface spot before the pit has developed show an early stage of atrophy of the cortical tubules belonging to the tubules destroyed in the lymphocytic foci. The atrophic tubules are smaller and more darkly stained than the normal, and their lumens are small or completely obliterated (Fig. 3). As the atrophy progresses these tubules become gradually converted into narrow epithelial cords, and in the final stages even these have disappeared entirely. Sections through pits such as are shown in Fig. 2 show complete atrophy of the tubules, but the shrunken glomeruli are easily identified (Fig. 5).

The tubules atrophy from the lymphocytic focus to the tip of the medullary pyramid as well as toward the surface of the kidney. This is easily seen by the study of serial sections of such a kidney as is shown in Fig. 2. The scar is readily traced from the surface pit down through the medulla to the tip of the pyramid. In the medulla it stands out very prominently as a band of tissue from which the tubules have disappeared (Fig. 4). The end-result of the lymphocytic infiltration at the cortico-medullary junction is the formation of a wedge-shaped scar with its apex at the tip of the pyramid and its base at the surface of the kidney. The lymphocytes usually occur only near the center of the wedge. That this is the shape of the lesion can be determined in any case by the study of serial sections.

We are not dealing therefore with a diffuse interstitial nephritis such as occurs in diphtheria and scarlet fever, but with discrete lymphocytic foci which cause atrophy of the entire tubule system by destroying its central portions. In severe cases where the foci fuse the appearance of acute interstitial nephritis is simulated.

The gross appearance of the kidneys in the late stages depends on the number of lymphocytic foci originally present. There may be only 2 or 3 pits, or the pits may be so closely set that the kidney has a shrunken contracted appearance. These severe extensive cases have given rise to the view that spontaneous nephritis in the rabbit is comparable to the contracted kidney of man, but this is not a correct interpretation. The most common form of chronic nephritis in man is a glomerulo-nephritis in which the primary injury is to the glomerulus, and the atrophy of the tubule is subsequent to the destruction of this structure. In the human arteriosclerotic kidney areas of tissue, of small or large size, undergo atrophy because of sclerosis of the nutrient artery. In spontaneous nephritis in the rabbit neither of these conditions obtains, but the atrophy results from destruction of a part of the tubule by an inflammatory exudate. The only form of human nephritis in any way comparable to rabbit nephritis is a form of focal infection in the kidney characterized by abscesses or diffuse inflammatory areas, and due to hematogenous infection. It is often referred to as "excretory" nephritis, or excretory pyelonephritis when the infection passes on into the pelvis of the ureter.

It is clear, therefore, that the successful solution of the problem of spontaneous nephritis in the rabbit will not necessarily throw any light on the problems of human nephritis. As far as we have observed spontaneous nephritis in animals other than the rabbit is similar to rabbit nephritis, but we have not studied other animals extensively enough to warrant a general statement. Le Count[7] described a case of chronic glomerulo-nephritis in a rabbit, and MacNider[8] reported this form as common in dogs. The important problem for experimental pathology in this connection is the production of a chronic glomerulo-nephritis.

Spontaneous nephritis frequently results in recovery. This is especially true in cases where the infection is not very extensive. Albumen disappears from the urine and the functional test is normal, but the pits on the surface persist. There is no attempt at regeneration of the scar tissue. In many cases, however, there is a progressive destruction of renal tissue. Death usually results from toxemia and intercurrent diseases rather than from renal insufficiency.

## URINARY FINDINGS

Albuminuria is usually demonstrable in the active stages of the disease. It may be intermittently present in mild cases, and it disappears entirely after healing. This last point is of considerable importance in experimental work. We have found deeply pitted and scarred kidneys at operation in several animals which had normal urine and normal phthalein excretion just before the operation. The presence of albumin in the urine does not necessarily mean that the rabbit has nephritis of the type described above. Our records show seven animals with albuminuria in which necropsy revealed only slight tubular injury in the kidney, the cause of which could not be clearly established. It is well known that albuminuria may be produced by a large variety of renal irritants. Casts are not constantly present.

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[7] Jour. Infec. Dis., 1914, 15, p. 389.

[8] Jour. Med. Research, 1916, 34, p. 177.

## EXPLANATION OF PLATE

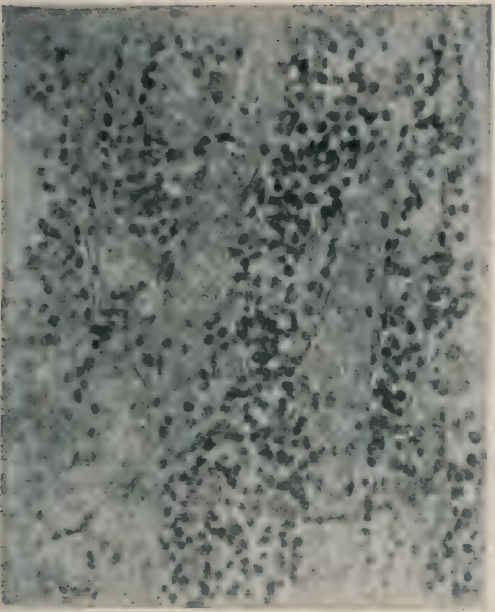
Fig. 1.—Photomicrograph. Area of lymphocytic infiltration at the cortico-medullary junction. Some tubules are already destroyed. This is the earliest type of lesion in spontaneous nephritis.

Fig. 2.—Photograph. Kidney showing numerous deep pits due to atrophy of cortical areas. The atrophic areas were produced by lymphocytic infiltrations at the cortico-medullary junction.

Fig. 3.—Early stage of atrophy of convoluted tubules due to destruction of their corresponding collecting tubules in a lymphocytic focus at the edge of the medulla. (The tubules in the lower left hand part of the figure are normal.)

Fig. 4.—Scar in medulla. This was traced in serial sections and found to connect with a surface pit such as is shown in Fig. 5.

Fig. 5.—Cortical pit (scar) showing complete disappearance of all the tubules. The glomeruli are still present. This is a healed lesion.



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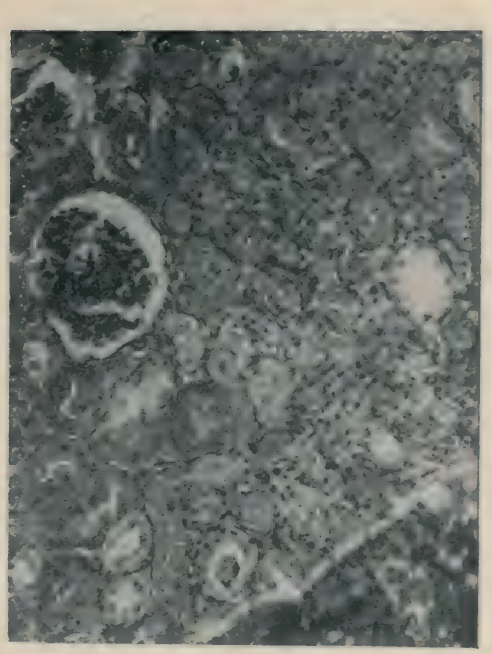

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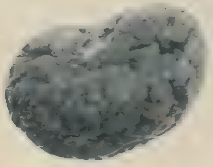

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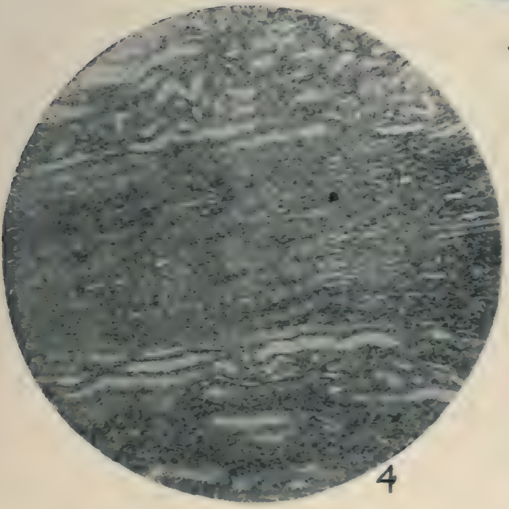

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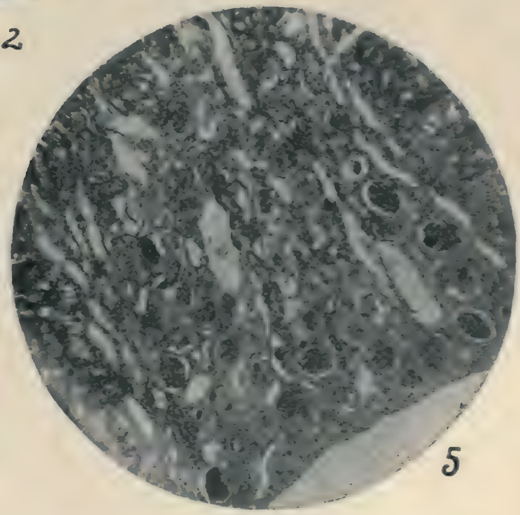

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### RENAL FUNCTION

The phenolsulphonephthalein test shows no change except in severe cases. The lowest 2-hour excretion we have recorded is 20%. When the phthalein test was reduced to any considerable degree extensive renal involvement was always present. Healed cases gave a high excretion, even though the scarring was considerable.

### DEVELOPMENT OF NEPHRITIS IN CONTROL ANIMALS

We have records of two rabbits with normal urine in which exploratory operation showed the surfaces of the kidneys smooth. These animals were kept as controls for some other experiments. About three months after the exploratory operation they were found to have developed a typical spontaneous nephritis. This observation is of great importance in interpreting experimental results. It is easily possible for nephritis to develop during the course of long experiments independently of the experimental procedure.

Another type of lesion occasionally seen has been described by several authors (Le Count,[7] Fig. 11, p. 402). It consists of a wedge-shaped area composed of dilated tubules with flattened epithelium. We have not worked out the genesis of this lesion.

### ASSOCIATED LESIONS

In all cases of spontaneous nephritis there was an associated lymphocytic infiltration of the portal connective tissue of the liver. This chronic hepatitis is also present in many cases in which the kidneys are normal. About 75% of the livers examined by us (50 cases) showed portal infiltration in varying degrees. Organisms may usually be cultivated from these livers but they do not always correspond with those obtained from the kidneys.

We did not make an extensive routine examination of the heart muscles in our nephritic cases; but in three rabbits in which large numbers of sections of heart muscles were examined we found foci of lymphocytes similar to those described by Longcope[9] and attributed by him to the action of foreign protein. It is probable that this form of myocardial infection is frequently associated with spontaneous nephritis.

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[9] Jour. Exper. Med., 1915, 22, p. 793.

### RELATION OF SPONTANEOUS TO EXPERIMENTAL NEPHRITIS

There has been no confusion of spontaneous with experimental acute nephritis since the two lesions are entirely different; but the natural disease has seldom if ever been sharply differentiated from chronic lesions supposedly produced by experimentation. As indicated in preceding paragraphs no observer has offered any convincing evidence that the lesion supposedly produced differs anatomically from the spontaneous disease. In fact, the illustrations accompanying many articles correspond entirely with the appearances of the natural disease. The usual argument is that a lesion occurring much oftener in experimental than in control animals must be due to the experimental procedure (O'Hare;[10] Dickson;[11] Christian, Smith and Walker;[2] Longcope[12]). But other explanations of these results are possible. 1. A spontaneous nephritis may develop during the course of experiments lasting several months. The control animals were not kept in the laboratory for the same length of time under the same conditions. Klotz[13] admits this as a possible source of error in his work. 2. The use of renal irritants such as uranium nitrate may favor the development of an infection in the kidneys, because of the general lowered resistance caused by the drug or because injured renal tissue is more susceptible than normal. The investigators who used uranium did not make cultures of the kidneys to see if bacteria were present. We obtained a growth of bacteria from one uranium kidney.

Observers who apparently did not examine the urine at the beginning of each experiment, and there are several in this group, are open to the criticism that the animals may have had nephritis at the outset of the experiment.

When virulent bacteria were injected into the blood stream, as in the experiments of Le Count,[7] Klotz,[13] and Hartzell and Henrici[14] it is easily possible that they are entirely responsible for the resulting lesions. One would expect a lesion resembling spontaneous nephritis from this procedure. The acute glomerular lesions obtained with streptococci suggest a relationship between these organisms and human glomerulo-nephritis; but the other lesions which various experimenters have attributed to injected bacteria bear no anatomic resemblance to the human disease.

[10] Arch. Int. Med., 1913, 12, p. 49.
[11] Ibid., 1909, 3, p. 375.
[12] Jour. Exper. Med., 1913, 18, p. 678.
[13] Trans. Assn. Am. Phys., 1914, 29, p. 49.
[14] Jour. Am. Med. Assn., 1915, 64, p. 1055.

## SUMMARY

Spontaneous nephritis in the rabbit is a subacute or chronic infectious disease characterized in the early stages by lymphocytic infiltrations in the kidneys usually at or near the cortico-medullary junction.

The parts of the tubules included in each lymphocytic focus undergo atrophy, and subsequently there is atrophy of the tubule systems both proximally and distally to the lymphocytic foci. The end-result is the formation of wedge-shaped scars with their apices at the apex of the pyramid and their bases formed by pits on the surface.

In our experience nephritis is much more common in some groups of rabbits than in others. The sanitary environment seems to be important. The disease is especially apt to develop in animals kept in the laboratory for several months.

Albuminuria is present in the active stages of the disease, but is absent in healed cases. Unlike human chronic nephritis many cases recover.

The investigators who believe they have produced chronic nephritis experimentally have not had controls adequate to exclude the spontaneous disease, and the lesions described correspond closely with spontaneous lesions.

No chronic glomerulo-nephritis has been produced experimentally. There is no anatomic or pathogenetic relationship between spontaneous nephritis in the rabbit and human chronic glomerulonephritis.

# Studies in the Nitrogen Metabolism of Bacteria

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# STUDIES IN THE NITROGEN METABOLISM OF BACTERIA

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The experiments here recorded were undertaken with the idea of gaining some information, if possible, as to the materials which usually serve as a source of nitrogen to bacteria, the actual form in which the nitrogenous substances are absorbed, and whether all simple dialyzable protein decomposition products are of equal usefulness in building up the cell substance.

The bulk of investigations of proteolysis in the past has had to do with the identification or determination of end-products. The early researches using mixed cultures on putrescible materials, although not usually controlled as to the exact chemical constituents of the medium, or the strains of bacteria employed, showed that the formation of certain substances such as ammonia, hydrogen sulphid, skatol, and so forth is characteristic of putrefaction irrespective of the precise nature of the protein or the particular varieties of organisms present. Productive of much more knowledge of actual bacterial metabolism was the long list of researches using pure cultures and mediums of known composition, making tests to determine the nature of the compounds formed. The indol test is the best known and most useful result of the investigations carried out on peptones or other tryptophane-containing medium.

Herter and Broeck[1] using a 1% preparation of casein with B. proteus demonstrated the presence of primary amins, hydrogen sulphid, fatty acids, aromatic oxyacids, indol, and indol acetic acid. Nawiasky[2] compared the products used up by B. proteus on a medium rich in peptone with those assimilated by Vibrio Finkler, B. fecalis-alkaligenes, and B. mesentericus, and states that B. proteus attacks albuminoses much more actively than do the others, but that its action on peptones and creatin was less marked. Rettger[3] planted B. putrificus Bienstock, B. edematis maligni, B. anthracis-symptomatici, B. coli, and B. lactis-aerogenes on special egg-meat medium and tested for indol, skatol, phenol, aromatic oxyacids, skatol-carbonic acid, tyrosin, leucin, albumoses, peptones, tryptophane, hydrogen sulphid, and mercaptan, his con-

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[1] Jour. Biol. Chem., 1911, 9, p. 491.

[2] Arch. f. Hyg., 1908, 64, p. 33.

[3] Jour. Biol. Chem., 1906, 2, p. 71.

clusion being that only the obligate anaerobes cause true putrefaction. Later with Newell[4] he tried 26 strains of B. proteus on egg-meat medium, blood fibrin suspended in broth, and Uschinsky's medium and concluded that, while actively proteolytic, B. proteus does not form those end-products characteristic of putrefaction in the narrow sense of the word. Effront[5] studied B. proteus, B. sporogenes, B. mesentericus, B. butyricus, B. putrificus Bienstock, etc., in a similar manner on albuminoid mediums. Sasaki's[6] researches on the particular substances formed from glycyl-glycin, glycyl tyrosin, cystin and tyrosin are of interest and value as pictures of a certain stage of protein degradation, as are the works of Brasch[7] on glutamic acid, serin, and tyrosin.

While investigations like those mentioned are of diagnostic importance, and throw much light on the types of chemical activity of which a given bacterium is capable, a definite idea of the metabolism of an organism cannot be obtained from purely qualitative data or from a single quantitative determination of some particular product. For such purposes a series of quantitative determinations should be made on a favorable medium, under conditions of vigorous normal growth. Many factors enter into the nature and amount of decomposition products formed: first, the availability of the nitrogenous compounds present, depending on whether or not the organism in question is capable of elaborating a ferment to digest them, the molecular point of attack of the enzyme, whether intermediate products are present, and, if so, what and in what quantity, what proportion of the split molecules are resynthesized into bacterial protein and which are left in the medium, and whether or not some of the compounds present are assimilated in preference to others. In the case of any particular end-product it is desirable to know whether it represents a portion of the protein molecule unattacked by the bacteria, a true cell excretion, or something set free by the death of the cell or by autolysis. Any systematic investigation of the proteolytic activity of an organism should include at least the following points:

1. Determination of the amounts present of certain available food substances.

2. Periodic determination of the amounts of one or more decomposition products used as a measure of proteolysis.

3. Allowances for such factors as rate of increase in numbers, presence of other non-nitrogenous foodstuffs, presence of decomposi-

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[4] Jour. Biol. Chem., 1912, 13, p. 341.

[5] Monit. Scient. Quesneville, 1911, I, p. 489.

[6] Biochem. Ztschr., 1912, 41, p. 174; 1912, 47, pp. 462-472; 1914, 59, p. 429; Sasaki. Takaoki and Otsuka: Biochem. Ztschr., 1912, 39, p. 208.

[7] Biochem. Ztschr., 1909, 18, p. 380; 1909, 22, p. 403.

tion products toxic to the cells or inhibitory of their activity, and products of autolysis of dead cells.

The infinite complexity of proteins, their delicate susceptibility to change under the influence of chemical and physical agents used for their isolation and purification, and the purely technical difficulties involved in an attempt to avoid accidental contaminations while preserving optimum conditions of growth as well as the refinements of chemical technic necessary to quantitative determination, have tended to discourage this type of research, with the result that there are comparatively few such studies on record, all more or less incomplete.

Among the end-products of putrefaction which have been determined quantitatively as a measure of proteolysis, ammonia has been the most common. Effront[8] made use of it in his studies on yeast. Emmerling and Reiser[9] stated that at least 25% of the nitrogen in gelatin was converted into ammonia by B. fluorescens-liquefaciens, while Arnaud and Charrin[10] found that in three days B. pyocyaneus converted 91% of the nitrogen in asparagin into ammonia and that a 3 weeks' culture in gelatin contained ammonia to the amount of 70% of the total nitrogen. Boencke[11] and Kendall and Farmer[12] determined ammonia to check the degree of proteolysis in their studies on the protein-sparing influence of carbohydrate. While the information thus obtained is valuable, particularly in the case of the putrefactive saprophytes, the ammonia curve admits of varying interpretations unless checked by other determinations. Moreover, many pathogens do not liberate much ammonia during their short period of life in artificial cultures (Berghaus[13]). The quantitative determination of indol and of phenol, valuable as they are in the study of B. coli and similar organisms, has the same limitation.

At the other extreme of the metabolic process, the progress of proteolysis may be observed by systematic determinations of the amount of coagulable protein remaining in the culture medium. De Waele and Vendevelde[14] worked out these results with a variety of organisms planted on gelatin, milk, and casein broth in an endeavor to settle the question of the specificity of bacterial proteases. Bainbridge,[15] Rettger and Sperry[16] working with representative pathogenic and saprophytic strains on medium composed of mineral salts and pure protein (crystallized egg albumin, serum protein, alkali albumin, and edestin) have proven that such growth as may occur on solutions of unchanged proteins is due to the minute amounts of nitrogenous impurities present, and that in the absence of split-protein products the germs are unable to attack native albumin.

[8] Compt. rend. Acad. de sc., 1908, 146, p. 779.

[9] Ber. d. deutsch. chem. Gesellsch., 1902, Part 3, p. 700.

[10] Compt. rend. Acad. de sc., 1891, 112, p. 755.

[11] Arch. f. Hyg., 1911, 74, p. 81.

[12] Jour. Biol. Chem., 1912, 12, p. 13; 1912, 13, p. 63:

[13] Arch. f. Hyg., 1908, 64, p. 1.

[14] Centralbl. f. Bakteriol., I, O., 1903, 39, p. 353.

[15] Jour. Hyg., 1911, II, p. 341.

[16] Am. Jour. Physiol., 1902, 8, p. 284.
Sperry: Report at Soc. Am. Bact., Montreal, Dec. 31, 1913.
Sperry and Rettger: Jour. Biol. Chem., 1914, 20, p. 445.

The quantitative determination of amino-acids is particularly useful, especially if checked by comparison with the amount of destruction of the protein or split-protein which is their source, or, in experiments using the amino-acids as a source of nitrogenous food, estimations of the ammonia which is their fate (Nawiasky[17]). Until very recently Sörensen's formol-titration method[18] has been the most in use. This procedure was followed by Rosenthal and Patai[19] who planted virulent and avirulent strains of staphylococcus, streptococcus and B. coli on broth. With all three of these organisms they observed an initial sharp rise of amino-acid, followed by a more gradual rate of increase. Meserintsky[20] observed a progressive augmentation of the amount of amino-acid present in a 10% gelatin culture of B. prodigiosus, which, however, represented only a portion of the non-coagulable nitrogenous material present. Frouin and Ledebt,[21] working with B. coli, B. typhosus, B. dysenteriae and V. cholerae on a solution of non-nitrogenous mineral salts enriched with amino-acids were unable to detect any decrease during the first 24 hours of growth, though the diminution became apparent later. Kendall, Day and Walker[22] attempted to use Sörensen technic in addition to determinations of ammonia, total nitrogen and acidity in their work on the protein-sparing action of glucose, but abandoned the amino-acid estimation because "the results furnished no information of importance."

The more delicate and accurate method of Van Slyke[23] now makes it possible to obtain much more significant estimation of the progress of proteolysis, since the micro-apparatus is accurate to 0.005 milligram of nitrogen.

This method was used by Sears[24] on peptone, broth, and gelatin cultures of a great variety of organisms. He also determined ammonia and creatinin. While some of his cultures showed a gradual increase in amino-nitrogen, the majority exhibited fluctuating rises and falls indicating in his opinion that amino-acids were formed and broken continuously during the life of the culture.

To arrive at a more nearly complete understanding of the nitrogen metabolism of bacteria it would seem valuable not only to apply such a series of tests to growth on peptone-containing mediums, but also to study the amino-acid content of cultures on some material which contains native albumin. This is particularly true of the pathogens, many of which do not thrive on plain peptone or broth. Moreover, their behavior in a medium closely resembling blood-serum or some other body fluid is well worth detailed consideration, both for the insight it might give into the process of infection, and the possibility of the usefulness of such data in attacking the chemical side of problems of immunity. Ascitic fluid is more similar to blood serum than any other material which can easily be obtained sterile in sufficient quantity for such purposes. Accordingly, determinations have been made on B. typhosus, B. proteus, B. pyocyaneus, and staphylococcus grown on broth, on pure ascitic fluid, and on mixtures of equal quantities of the two. In order to rule out the protein-sparing properties of carbohydrates as much as possible in the interests of simplicity, sugar-free broth was used. Such broth may contain considerable amounts of amino-acid determined by Van Slyke's method in addition to the peptones, but no coagulable albumin or carbohydrates. In ascitic fluid, on the other hand, the amount of amino-acid and other protein decomposition products is small, while the nitrogen present as coagulable protein is considerable. A small amount of carbohydrate is probably present, but no tests for it were made. A mixture of equal parts of broth and ascitic fluid represents a middle ground between these conditions. In the experiments recorded in tables 4 and 10 ascitic fluid was distributed in equal amounts (10 c c) in a series of sterile test tubes, and each inoculated with a loopful of broth culture. At regular intervals the amino-acid content of one tube was determined, the loss by evaporation being carefully made up with distilled water. While this method has the advantage of permitting determinations to be made without danger of contamination of the material destined for later analysis, there is always the possibility of a considerable variation in the seeding of the different tubes or even in the samples of ascitic fluid. The rest of the work was done with flasks containing a quarter of a liter or more of the medium. Tight fitting rubber caps over the cotton stoppers protected against evaporation. At intervals samples were removed with sterile pipettes after shaking the flask to render the medium homogeneous. These were centrifuged and the supernatant liquid (practically, though probably not absolutely, free of bacterial cells) was tested for the amount of amino-acid present, and in some cases for coagulable protein. In cases where numbers of bacteria present are given, they were determined by counting agar plates made with the customary quantitative precautions. The Kjeldahl determinations were run in duplicate, and the results averaged if they did not check but showed a difference of less than 0.5 c c in the titration. Figures showing a wide variation were discarded. Two Van Slyke determinations were made routinely, checked by a third in case the amounts of nitrogen liberated did not agree within 0.03 c c.

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[17] Arch. f. Hyg., 1908, 66, p. 209.

[18] Biochem. Ztschr., 1907, 7, pp. 45 and 407.

[19] Centralbl. f. Bakterial., I, O., 1914, 73, p. 406.

[20] Biochem. Ztschr., 1910, 29, p. 104.

[21] Compt. rend. Soc. de biol., 1911, 70, p. 24.

[22] Jour. Am. Chem. Soc., 1913, 35, p. 1201.

[23] Jour. Biol. Chem., 1911, 9, p. 185; 1912, 12, p. 275; 1913, 16, p. 121; 1915, 23, p. 407.

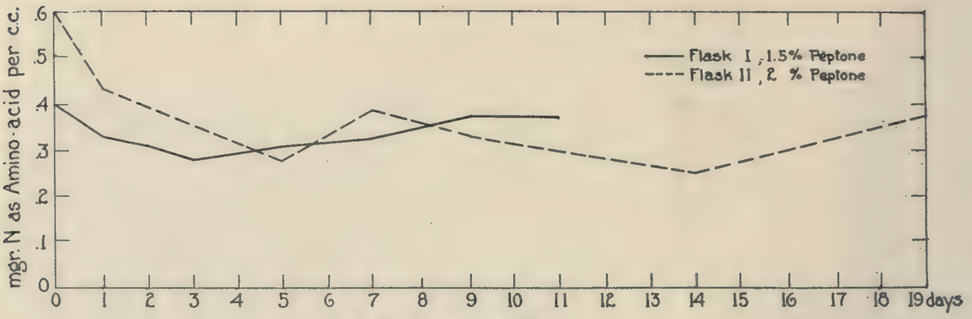
[24] Jour. Infect. Dis., 1916, 19, p. 105.

TABLE 1

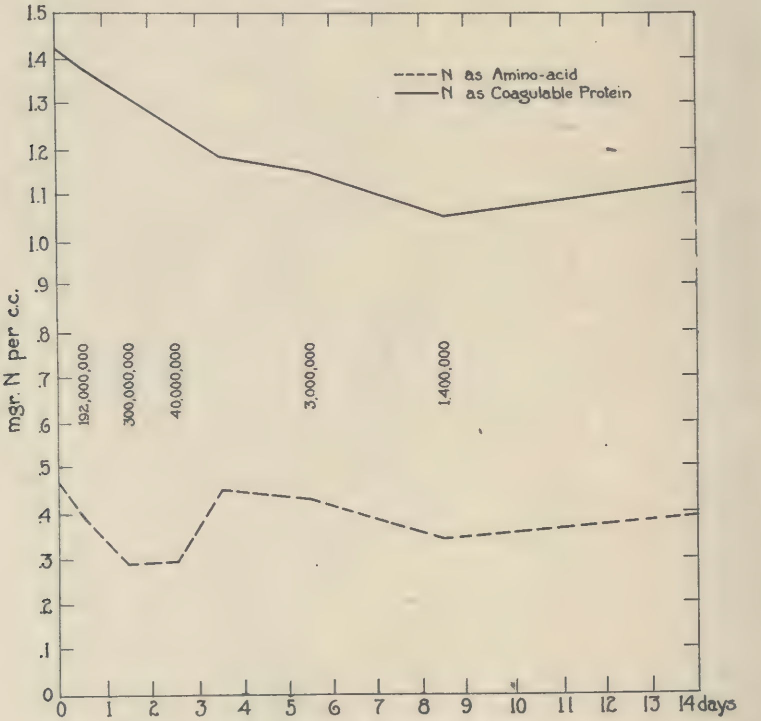
STAPHYLOCOCCUS

| | Milligrams N As Amino-Acid per C C in Broth | |
|---|---|---|
| Incubation | Flask 1 (2% Peptone) | Flask 2 (1.5% Peptone) |
| Sterile | 0.606 | 0.407 |
| 1 day | 0.438 | 0.333 |
| 2 days | ..... | 0.315 |
| 3 days | ..... | 0.280 |
| 5 days | 0.280 | 0.319 |
| 7 days | 0.390 | 0.328 |
| 9 days | 0.332 | 0.376 |
| 11 days | ..... | 0.369 |
| 14 days | 0.252 | |
| 19 days | 0.380 | |

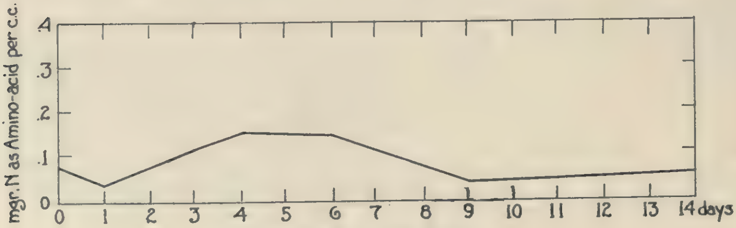
The staphylococcus cultures on broth (table 1, chart 1 a) show a drop to 0.29 mg. though flask 1 originally contained nearly half again as much amino-acid as flask 2. Flask 1 on the chart is shown as having reached this minimum two days later than flask 2 but this may well be due to the fact that no 3-day determination was recorded. In flask 2 there was a steady gradual rise after reaching the minimum until the point when it had to be discarded on account of contamination. Flask 1 shows fluctuations which are without significance when not checked by determinations of other substances.



a. On Sugar-free Broth.



b. On Sugar-free Broth + Ascitic Fluid.



c. On Pure Ascitic Fluid

CHART I. STAPHYLOCOCCUS.

On a mixture of broth and ascitic fluid (table 2 and chart 1 b) the amino-acid curve shows a decided fall during the period of most rapid multiplication, followed by a rise and later some fluctuation.

TABLE 2

STAPHYLOCOCCUS

Milligrams N per C C As Amino-Acid and As Coagulable Protein in a Mixture of Ascitic Fluid and Broth

| Incubation | Amino-Acid | Coagulable Protein | Bacteria per C C |
|---|---|---|---|
| Sterile | 0.468 | 1.420 | |
| 12 hours | 0.393 | 1.382 | 192,000.000 |
| 36 hours | 0.295 | 1.312 | 300,000,000 |
| 60 hours | 0.306 | .... | 40,000,000 |
| 84 hours | 0.445 | 1.189 | |
| 132 hours | 0.425 | 1.158 | 3,000,000 |
| 8½ days | 0.341 | 1.052 | 1,400,000 |
| 14 days | 0.390 | 1.134 | |

On pure ascitic fluid (table 3, chart 1 c) although the amount of amino-acid present to begin with was much smaller than in any of the other flasks, we observe the same initial drop followed by a slight rise and a subsequent fall.

TABLE 3

STAPHYLOCOCCUS

Milligrams N As Amino-Acid per C C of Pure Ascitic Fluid

| Incubation............ | Sterile | 1 day | 3 days | 4 days | 6 days | 9 days | 14 days |
|---|---|---|---|---|---|---|---|
| Milligrams N.......... | 0.067 | 0.033 | 0.112 | 0.145 | 0.143 | 0.033 | 0.054 |

Apparently this organism readily assimilates a part at least of the amino-acids found in each of these mediums but attacks the coagulable protein to some extent from the first. After about 3 days there comes a period when amino-acids accumulate, apparently due partly to autolysis and partly to the fact that the original content of assimilable amino-acid is exhausted, and only a portion of those liberated from the proteins, peptones, etc., can be used by the organism. The increase of coagulable protein after the 8th day may be due to the fact that, as the cultures grew older, it became increasingly difficult to get a clear supernatant fluid by centrifugation.

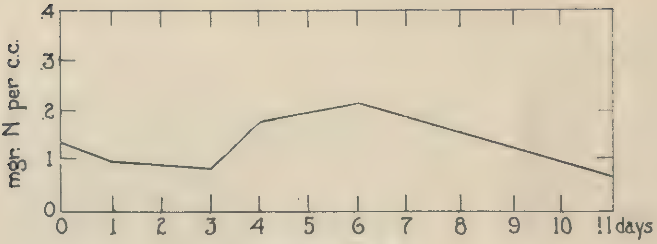
The results obtained in similar mediums with B. pyocyaneus are noticeably different. On pure ascitic fluid (table 4, chart 2 c), after an initial drop, a steady rapid rise in amino-acid is observed.

TABLE 4

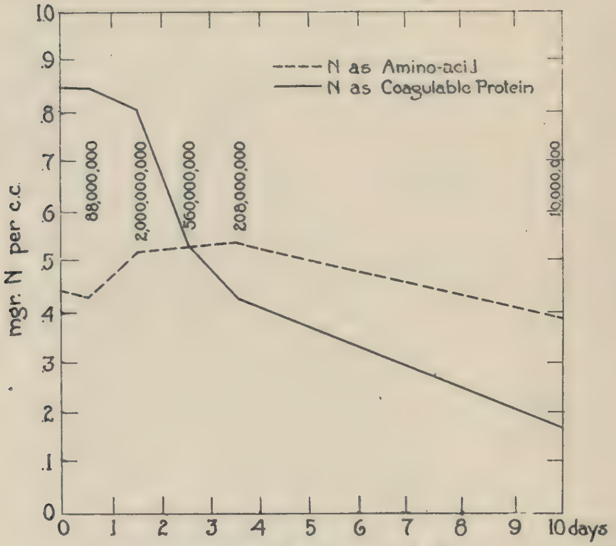
B. PYOCYANEUS

Milligrams N As Amino-Acid per C C of Ascitic Flud

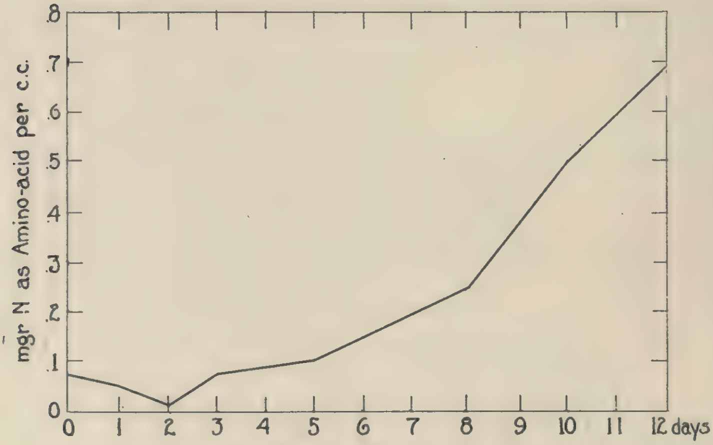
| Incubation........ | Sterile | 1 day | 2 days | 3 days | 5 days | 8 days | 10 days | 12 days |
|---|---|---|---|---|---|---|---|---|
| Milligrams N..... | 0.077 | 0.049 | 0.013 | 0.074 | 0.099 | 0.249 | 0.493 | 0.695 |



a. On Sugar-free Broth.



b. On Sugar-free Broth + Ascitic Fluid.



c. On Pure Ascitic Fluid.

Chart II. Bacillus Pyocyaneus.

On sugar-free broth, however, there is a variation (table 5, chart 2 a) which may be significant. After the original drop (which would perhaps be more noticeable if a 2-day determination had been made) and a subsequent increase between the 3rd and 6th days, there is an unmistakable drop shown on the 11th day. Apparently, after exhausting the favorite amino-acids present in the peptone, the organism attacks the previously unused amino-acid while in an abundance of available protein it continues uneconomically to select for synthesis the molecules best adapted to its needs.

### TABLE 5

B. PYOCYANEUS

| | Milligrams N As Amino-Acid per C C of Broth | | | | | |
|---|---|---|---|---|---|---|
| Incubation................ | Sterile | 1 day | 3 days | 4 days | 6 days | 11 days |
| Milligrams N.............. | 0.1384 | 0.098 | 0.086 | 0.184 | 0.215 | 0.066 |

On a mixture of broth and ascitic fluid (table 6, chart 2 b). the results are somewhat similar, except that the rise in amino-acid occurred earlier. Here, too, we have a decrease in amino-nitrogen after the fourth day, although there is destruction of protein going on at the same time. Curiously enough, the greatest destruction of protein took place after the maximum count had been passed and at a time when the free amino-acids were not noticeably increasing. The protein destruction might be attributed to the activity of the enzymes previously secreted by the bacteria but in that case an increase of amino-acid would be expected. The fact that the acids do not remain free in the culture can be explained on either of two suppositions: that the enzymes secreted were of more than one kind and could carry proteolysis beyond the amino-acid stage or, on the other hand, that cell division and consequently synthesis of amino-acid into bacterial protoplasm was proceeding at a maximum rate during this period, the apparent decrease in numbers being due to the fact that the death rate began to make itself felt at the point when the count was 2,000,000,000.

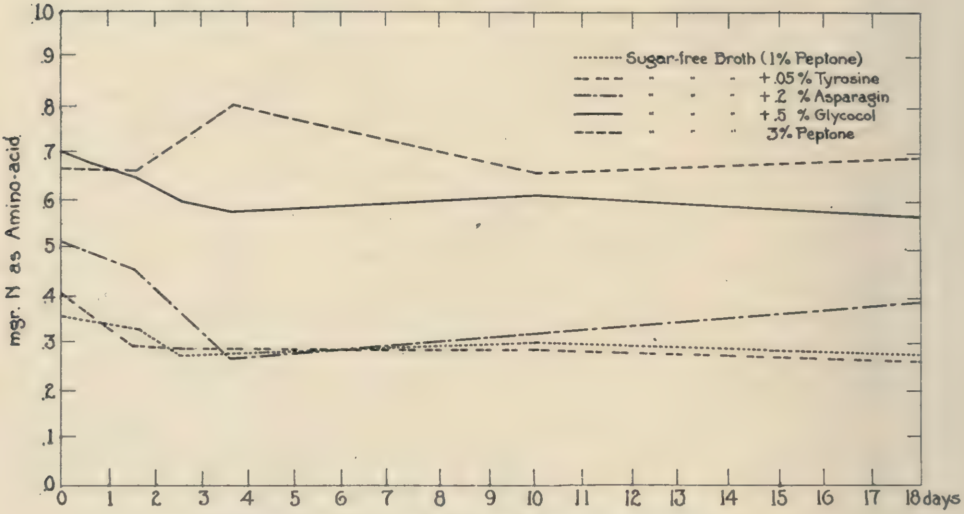
### TABLE 6

B. PYOCYANEUS

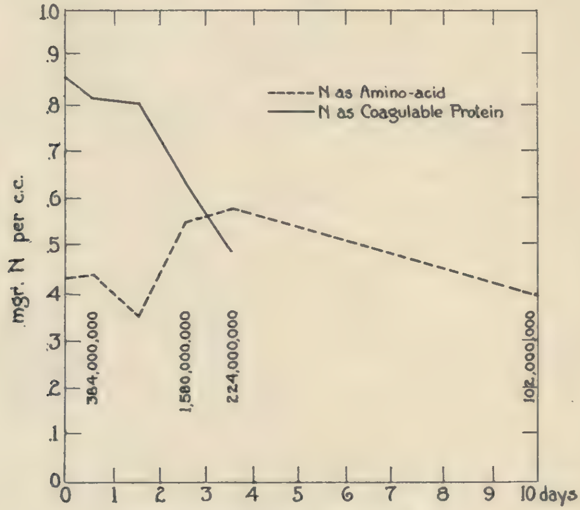
Milligrams N per C C As Amino-Acid and As Coagulable Protein on Ascitic Fluid + Sugar-Free Broth

| Incubation | Amino-Acid | Coagulable Protein | Bacteria per C C |
|---|---|---|---|
| Sterile | 0.441 | 0.846 | |
| 12 hours | 0.439 | 0.846 | 88,000,000 |
| 36 hours | 0.520 | 0.818 | 2,000,000,000 |
| 60 hours | ..... | 0.529 | 560,000,000 |
| 84 hours | 0.537 | 0.433 | 208,000,000 |
| 10 days | 0.386 | 0.172 | 10,000,000 |

In order to throw more light on this apparent preference for some protein-decomposition products instead of others, it seemed useful to investigate the effects of the addition of varying amounts and kinds of amino-acids to culture mediums. Accordingly, 0.1 c c of 48-hour broth culture of B. typhosus was inoculated into each of five flasks of sugar-free broth which differed only in that flask 1 was ordinary sugar-free broth containing 1% peptone, flask 2 contained in addition 0.05% tyrosin, flask 3 0.2% asparagin, flask 4 0.5% glycocol, and flask 5 extra peptone making a strength of 3%. The results are given in table 7 and represented graphically in chart 3 a.



a. On Sugar-free Broths of different Amino-acid Content.



b. On Sugar-free Broth + Ascitic Fluid.

CHART III. BACILLUS TYPHOSUS.

TABLE 7

B. TYPHOSUS

| | Milligrams N As Amino-Acid per C C on Broths of Varying Amino-Acid Content | | | | |
|---|---|---|---|---|---|
| Incubation | 1% Peptone | 0.05% Tyrosin 1% Peptone | 0.2% Asparagin 1% Peptone | 0.5% Glycocol 1% Peptone | 3% Peptone |
| Sterile | 0.357 | 0.401 | 0.520 | 0.715 | 0.678 |
| 16 hours | ..... | 0.343 | | | |
| 18 hours | ..... | .... | ..... | 0.686 | |
| 36 hours | 0.321 | 0.295 | | | |
| 38 hours | ..... | ..... | 0.452 | 0.651 | 0.667 |
| 60 hours | 0.277 | 0.282 | ..... | 0.605 | |
| 90 hours | ..... | ..... | 0.272 | 0.577 | 0.806 |
| 10 days | 0.309 | 0.315 | ..... | 0.619 | 0.663 |
| 18 days | 0.277 | 0.265 | 0.259 | 0.567 | 0.686 |

In the flask of plain sugar-free broth we note a gradual decrease in amino-acid during the first two days. This period covers the lag-phase and the period of most rapid multiplication, during which it uses those acids present in the medium which the organism is especially able to assimilate. The fact that no great change was apparent at the times when subsequent tests were made would seem to indicate that the peptone in the culture was being used, and that the resulting decomposition products were either synthesized into bacterial protoplasm or else carried beyond the amino-acid stage. However, there seems to be a certain amount of amino-acid present which remains untouched, doubtless being unsuitable as nutriment for the cells. In the tyrosin flask the same level is reached and maintained after 60 hours, indicating that the extra foodstuff in that form was readily assimilated. The flask with asparagin shows the same process in a more striking degree. It is unfortunate that no 60 hour test was made to determine whether the asparagin was used up in that length of time, or really required 3½ days to reach the dead level as the chart seems to show. The flask which contained a considerable amount of glycocol shows a similar absolute decrease, but the level maintained is higher than that of the other flasks by just about the amount of glycocol added. (The difference in amino-acid nitrogen between the plain broth flask and the one with glycocol was 0.358 mg. per c c when sterile, and 0.354 mg. after 10 days' incubation.) This apparently indicates that glycocol is not assimilated by B. typhosus when other sources of nitrogen are present. In the 3% peptone flask there is very little decrease during the first 1½ days, indicating that the free amino-acid used up is balanced by the fact that some is being set free from the peptone. After this, apparently the peptone destruction sets free more acid than is used up, but later still these are synthesized or more likely broken down into simpler substances. (Sears found that B. typhosus formed considerable amounts of ammonia on peptone solution.)

These results are confirmed and explained by an experiment using a medium consisting of mineral salts, glycerol and a pure amino-acid. Absolutely no visible growth of B. typhosus could be determined in such a medium with glycocol as a sole source of nitrogen, although it grew well on this medium when other amino-acids were introduced, and several other bacteria produced a heavy growth on the glycocol. In the typhoid-glycocol culture tubes there was no appreciable diminution of the amount of amino-acid present during 3 weeks, though the bacilli introduced remained viable throughout the period of observation.

The results obtained with the same strain on a mixture of sugar-free broth and ascitic fluid (table 8, chart 3 b) are of interest in connection with the first experiment.

TABLE 8

B. TYPHOSUS

| Milligrams N per C C As Amino-Acid and As Coagulable Protein in a Mixture of Broth and Ascitic Fluid | | | |
|---|---|---|---|
| Incubation | Amino-Acid | Coagulable Protein | Bacteria per C C |
| Sterile | 0.430 | 0.859 | |
| 12 hours | 0.439 | 0.818 | 384,000,000 |
| 36 hours | 0.354 | 0.805 | |
| 60 hours | 0.551 | 0.639 | 1,580,000,000 |
| 84 hours | 0.581 | 0.488 | 224,000,000 |
| 10 days | 0.386 | Lost | 102,000,000 |

Here the protein is attacked from the first, and there is even a slight rise in amino-acid during the first 12 hours, then a considerable fall in the next 24, followed by a rapid rise in free amino-acid, and great destruction of protein continuing even a little after the maximum count was reached. Then ensues a period during which the amino-acids are gradually used up by the germs.

The two flasks of ordinary sugar-free broth inoculated with B. proteus (table 9, chart 4 a) indicate that, after the first drop due to the utilization of the amino-acids present, very little of the amino-acid broken from the peptone is left free in the medium, and the accumulation is not great.

TABLE 9

B. PROTEUS

| | Milligrams N As Amino-Acid per C C of Broth | |
|---|---|---|
| Incubation | Flask 1 | Flask 2 |
| Sterile | 0.329 | 0.329 |
| 1 day | 0.239 | 0.233 |
| 2 days | 0.191 | 0.202 |
| 6 days | 0.230 | 0.235 |
| 8 days | 0.282 | 0.282 |

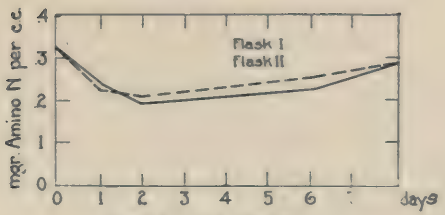
On undiluted ascitic fluid (table 10, chart 4 c) this is even more marked. Unfortunately, determinations of coagulable protein were not made, but the idea that the small amount of free amino-acid is due to thoroughness of decomposition rather than to lack of protein is strengthened by the fact that this was the same sample of fluid on which B. pyocyaneus set free 0.695 mg. per c c in 12 days.

TABLE 10

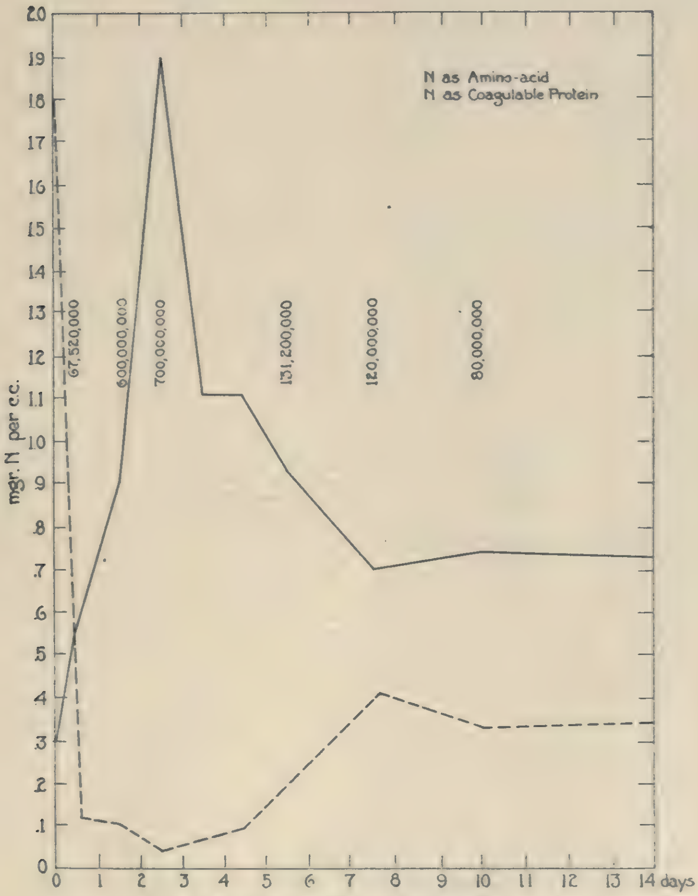
B. PROTEUS

| Milligrams N As Amino-Acid per C C of Pure Ascitic Fluid | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Incubation | Sterile | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days | 12 days |
| Milligrams N | 0.077 | 0.060 | 0.054 | 0.043 | 0.026 | 0.031 | 0.054 | 0.032 | 0.026 |

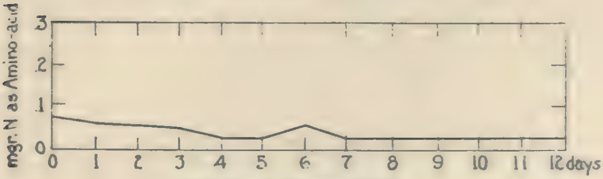
On the mixture of broth and ascitic fluid (table 11, chart 4 b) the striking destruction of coagulable protein, and the very large amount of amino-acid liberated in the first 2½ days apparently indicates that this organism attacks both protein and peptones whenever they are present, indiscriminately, and uneconomically in the case of a medium rich in both. Later much of this surplus amino-acid was used up.



a. On Sugar-free Broth.



b. On Sugar-free Broth + Ascitic Fluid.



c. On Pure Ascitic Fluid.

CHART IV. BACILLUS PROTEUS.

TABLE 11

B. PROTEUS

| | Sugar-Free Broth Plus Ascitic Fluid | | |
|---|---|---|---|
| Incubation | N per C C As $NH_2$ | N per C C Coagulable Protein | Count |
| Sterile | 0.307 | 1.807 | 0 |
| 14 hours | 0.575 | 0.119 | 67,520,000 |
| 36 hours | 0.900 | 0.105 | 600,000,000 |
| 2½ days | 1.980 | 0.049 | 700,000,000 |
| 3½ days | 1.175 | | |
| 4½ days | 1.173 | 0.091 | |
| 5½ days | 0.934 | ..... | 131,200,000 |
| 7½ days | 0.707 | 0.412 | 120,000,000 |
| 10 days | 0.743 | 0.337 | 80,000,000 |
| 2 weeks | 0.732 | 0.348 | |

In this flask as in the staphylococcus flask already mentioned, it was almost impossible to obtain a clear fluid by centrifugation after the 1st week, and this probably accounts for the rise in coagulable protein noted on the 7th day.

It is interesting to compare the results on the flasks of broth reinforced with various amino-acids (table 12, chart 5) with the results on plain broth as well as with the behavior of B. typhosus.

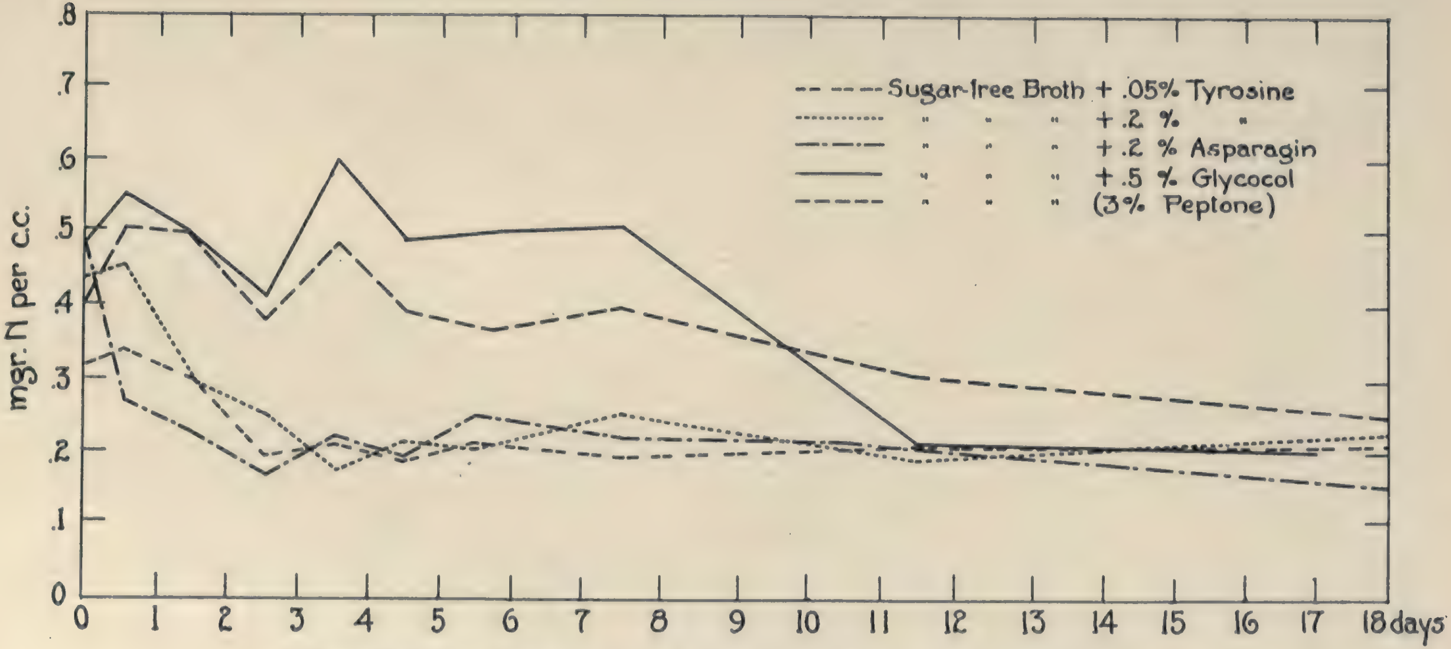
TABLE 12

B. PROTEUS

| | 0.05% Tyrosin | 0.2% Tyrosin | 0.2% Asparagin | 0.5% Glycocol | 3% Peptone |
|---|---|---|---|---|---|
| Sterile | 0.327 | 0.440 | 0.469 | 0.480 | 0.402 |
| 12 hours | 0.345 | 0.449 | 0.276 | 0.556 | 0.503 |
| 36 hours | 0.309 | 0.292 | 0.220 | 0.496 | 0.499 |
| 60 hours | 0.199 | 0.254 | 0.165 | 0.417 | 0.386 |
| 84 hours | 0.209 | 0.175 | 0.219 | 0.602 | 0.481 |
| 4½ days | 0.188 | 0.210 | 0.190 | 0.485 | 0.397 |
| 5½ days | 0.212 | 0.201 | 0.257 | 0.491 | 0.372 |
| 7½ days | 0.199 | 0.255 | 0.221 | 0.518 | 0.408 |
| 10½ days | ..... | ..... | 0.219 | | |
| 11½ days | 0.209 | 0.188 | ..... | 0.208 | 0.297 |
| 17 days | ..... | . ... | ..... | 0.204 | |
| 18 days | 0.209 | 0.222 | 0.155 | ..... | 0.255 |

Here only one flask showed an initial fall in amino nitrogen content, namely, that containing asparagin. This substance is apparently assimilated with considerable vigor at the same time that the peptone is being broken down, and the "dead level" of useless amino-acid is reached in 2½ days. In the case of tyrosin the amounts set free are in excess of the amounts liberated during the first twelve hours but in a few days the content is about the same as that of the asparagin flask. The glycocol flask shows a very different curve from that produced with B. typhosus. Here we have an initial rise due to the attack on peptone, followed by fluctuations and a 3-day period of no great change, and then a rapid fall between the 7th and 11th days, when the "dead level" was reached. In the 3% peptone flask the initial increase is more marked than in any of the others, which is what might be expected, considering the readiness of the organism to attack such substances, and the fact that there is three times as much peptone present as in each of the other four flasks.

The results on the synthetic medium (mineral salts, glycerol and a single amino-acid) agree with these findings. While B. proteus did not use up



On Sugar-free Broths of different Animo-acid Content.

CHART V. BACILLUS PROTEUS.

glycocol as rapidly as some other bacteria did, it grew fairly well, proving that it can derive nitrogen from this acid if "starved to it." These peculiarities seem worthy of further study. It was thought possible that this difference might have some bearing on the liquefaction of gelatin, which yields a large percentage of glycocol on hydrolysis. However, several bacteria (B. paratyphosus, B. coli, B. fecalis-alkaligenes) incapable of liquefying ordinary gelatin mediums, thrive on these glycocol solutions. Investigations using a variety of amino-acids for a considerable number of organisms promise interesting results, and are well under way.

## SUMMARY

Four organisms were studied, two of which are strongly proteolytic on ordinary mediums (B. proteus and B. pyocyaneus), one moderately so (staphylococcus), and one (B. typhosus) which does not show the marked activity of the others. On mediums containing ascitic fluid all four destroyed considerable amounts of coagulable protein during the first 36 hours. B. pyocyaneus was the only one which did not do so in the first 12. In the case of the staphylococcus this digestion proceeded at a fairly uniform rate; B. typhosus and B. pyocyaneus apparently did not attack the coagulable protein vigorously until the more easily assimilable substances had been used; B. proteus seems to attack all the nitrogenous materials at once without discrimination. B. proteus and B. typhosus could both assimilate tyrosin and asparagin; B. proteus could make use of glycocol; B. typhosus could not.

## DISCUSSION AND CONCLUSIONS

Evidently, the ability of a given cell to assimilate amino-acids does not result directly from their simplicity of structure and solubility in water; otherwise glycocol, which is the simplest and is readily soluble, would be the most easily used. We have been led to believe that the intracellular proteins synthesized by bacteria are specific, in view of various phenomena connected with immunity. It seems reasonable to suppose that this individuality may rest to some extent on the ability to absorb certain substances characteristic of those proteins. Sperry and Rettger found that pure crystallized proteins cannot be attacked by bacteria in the absence of decomposition products; experiments made in this department by Diehl seem to indicate that bacteria will not elaborate a ferment against a certain protein if grown on a medium containing none of the amino-acids found in that protein. The idea of individual requirements in amino-acid by various kinds of bacteria lends color and significance to these observations. We can think of the free amino-acids present in a culture containing a protein and its split products as both "building stones" in the protein molecule, and as possible "stepping stones" for the bacteria. Then the avidity with which an organism attacks a protein would be in direct proportion to the amount and variety of free amino-acids present which are represented in the structure of the protein molecule and which that particular kind of cell can assimilate. If only a few acids fulfilling both these requirements are found in the medium, the stimulus to proteolysis would not be great, at least until possibly a greater quantity and variety was set free by the first feeble attack. An organism exhibiting what might be called a catholicity of taste in amino-acids would have an increased number of approaches to the protein molecule. Further investigations will show whether or not one is justified in stating that B. proteus, for instance, is such an organism.

In the meanwhile we are certainly justified in concluding that the nature of the particular protein decomposition products present play a very important part in metabolism, as the power to assimilate a given amino-acid is not necessarly common to all bacteria, but is due to factors which may be absent in some varieties.

# THE SPECIFICITY OF BACTERIAL PROTEOLYTIC ENZYMES AND THEIR FORMATION

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Ever since the recognition of enzymes as the active agents in a large number of vital processes of both multicellular and unicellular organisms, much work has been done to determine their nature and properties. Through this we have learned much about them, but a great deal is still left unknown, mainly because it has been impossible to obtain them in a pure form. So at present we can study them only indirectly, that is, by investigating their properties and their modes of action.

One of the first properties assigned to amylases, lipases, and proteases was that of specificity. Even among the enzymes acting on the several carbohydrates a certain amount of specificity was observed; but the nature of this specificity could not be determined because it appeared that these ferments were inseparable from the bacterial cells. They are what we now know as endo-enzymes of bacteria. With the proteolytic enzymes, however, more work was possible because these are given off by the cells into the surrounding medium, and are therefore susceptible of isolation.

The action of these enzymes on the proteins offered a good opportunity for the study of specificity because of the great differences between the several types of proteins. If enzymes really be specific, then one would expect that no single enzyme would be able to act on all of the widely different types of proteins. On the other hand, it hardly seems probable that a different enzyme exists for each. For, if this were actually the case, digestive enzymes such as pepsin and trypsin would have to be considered not as units, but as combinations or mixtures of many distinct ferments. Such a conception does not seem probable, nor is it borne out by the experiments of Fermi[1] and others, who came to the conclusion that the proteolytic enzymes of the digestive juices are units and are not specific for individual proteins.

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Fermi,[1] in discussing this question, quotes the views of Fischer and Abderhalden. Their opinions are very similar and well worth repeating here. Emil Fischer states: "That most of our common ferments are mixed is very probable. But how far one should go in the differentiation of them is hard to say without experiment; and I must admit that most experiments which have been carried out in this connection possess but very little conclusive force. In any case, I believe that it is an exaggeration to assume for each protein that is attacked by pepsin and trypsin a special enzyme; for the necessary conclusion would be that one must assume for each glucosid a special enzyme, namely, for beta-methyl-glucosid and for beta-phenyl-glucosid. According to chemical analogies, I must hold this as being very improbable." Abderhalden expresses very much the same view when he says: "It is quite probable that pepsin and trypsin are not units, still, any sure proof of a gelatinase, casease, etc., is lacking in my conviction at the present time. So long as one is forced to work with unknown substrata and unknown ferments, sure conclusions are very hard to get."

In investigating this problem numerous proteins have been used to test enzyme action but gelatin has been more popular than any other. This is so chiefly because gelatin is easily prepared and any proteolytic action on it is discernible by direct observation. For this reason the digestion of gelatin has come to be looked on by many bacteriologists as an index of the proteolytic activity of bacteria, and in most cases it is quite satisfactory. Next to gelatin, casein probably has been most used, with variations of technic to determine the amount of its digestion. Hemoglobin, fibrin and other proteins have been tried with more or less satisfactory results.

## REVIEW OF THE LITERATURE

Pollak,[2] after much experimenting, was convinced, (1) that the gelatinases are not identical with trypsin and (2) that a specific gelatinase exists. He bases this latter conclusion on the fact that in immune serum he could demonstrate an antigelatinase, which would suspend the gelatinolytic activity of solutions but would leave uninfluenced the serolytic. Hattori[3] also claims to have demonstrated a specific gelatinase by the use of chemicals which would destroy the albumino-, sero- and fibrino-lytic powers of a solution but would leave unimpaired the gelatinolytic power.

On the other hand, Duclaux[4] thinks that the gelatinases and caseases are identical, and also assumes the identity of both of these with trypsin. And

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[1] Centralbl. f. Bakteriol., 1, O., 1913, 68, p. 433.

[2] Cited by Marras (Hofmeister's Beitr., 1905), 6, p. 95.

[3] Cited by Marras, Arch. internationale de pharmacol., 1908, 18, p. 255.

[4] Microbiologie, 2, p. 618.

Fermi,[5] after extensive work with ferments obtained from animals and plants, including bacteria, concludes that the proteolytic enzymes are identical with trypsin. He found that, when fibrinases and caseases were present, gelatinases were also present; but that some bacteria which produce gelatinases do not produce fibrino- and caseino-lytic enzymes. From these findings he draws the conclusion that the same enzyme acts on all proteins, but that different proteins show various degrees of resistance.

De Waele and Vandevelde[6] also believe that the differences in bacterial proteolysis are differences of intensity only. They state that on the different nitrogenous mediums all bacteria act quite similarly and that all organisms which liquefy gelatin also vigorously attack casein. To them this parallelism indicates that the liquefaction of gelatin and the solution of casein are due to one and the same ferment. However, they also find that bacteria produce various amounts of proteolytic ferments and that these ferments differ somewhat in their action; but that these differences depend not so much on the bacteria as on the conditions and environment under which the enzymes are produced.

By the use of blood agar Eijkmann[7] demonstrated an enzyme liberated by B. diphtheriae which was hemolytic but not proteolytic; and one liberated by a coccus from the air which digested gelatin but did not affect hemoglobin. To account for these differences he suggests several possibilities: (1) That different kinds of proteolytic enzymes exist, one of which will most readily act on blood and another on gelatin and (2) that some metabolic products of the bacteria other than the ferments are responsible for some of the actions. The latter possibility is one which has not been mentioned by any other workers and is decidedly the less probable of the two. He also utilized agar-elastin plates and on these observed the action of an enzyme that seemed to him identical with "the peptonizing ferment which liquefies gelatin." But, he states that there was always some incongruity in the clearing of the elastin and the liquefaction of the gelatin. This is exactly what one should expect, since gelatin and elastin though very closely related chemically are nevertheless not identical.

Mavrojannis,[8] working with 8 types of bacteria, found that 5 of these digested gelatin to the stage of gelatoses; while the other 3 would carry them through to gelato-peptones. The gelatoses can be resolidified by means of formol while the gelato-peptones are unaffected by it. From these results he concluded that at least two kinds of ferments were responsible for the breaking down of gelatin, and that each of these was specific. These results, however, were soon refuted by Bogoluboff[9] and Tiraboschi;[10] but the work remains of some value because it helped to stimulate other investigations along the same lines. The latter authors found that the production of gelatoses or of gelato-peptones depends not so much on the type of ferment as on the temperature, the reaction of the medium, and the length of time which the ferments are allowed to act.

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[5] Centralbl. f. Bakteriol., 1913, 1, O., 68, p. 433; 1913, 69, p. 465.

[6] Centralbl. f. Bakteriol., 1, O., 1905, 39, p. 353.

[7] Centralbl. f. Bakteriol., I, 1901, 1, p. 841.

[8] Compt. rend. Soc. de biol., 1903, 55, p. 1605.

[9] Bull. de l'Instit. Pasteur, 1907, 5, p. 429.

[10] Bull. de l'Instit. Pasteur, 1905, 3, p. 922.

The review of the literature shows a great diversity of results and conclusions, but gives the general impression that proteolytic enzymes are probably not specific. Still such a conception leaves unexplained some of the phenomena which these enzymes often exhibit. The statement is frequently encountered that the conditions and environment greatly influence the formation and properties of bacterial enzymes. Of all the factors that are included under the conditions and environment of enzyme formation, the composition of the medium is probably the most important.

In this connection the question suggests itself as to whether enzymes are preformed in the bacterial cell or whether they are produced by the organism only when the need for them arises. From our knowledge of vital processes in general it seems more probable that the bacterial cell should be endowed with the ability to elaborate ferments than that it should be a storehouse for the many ferments of which it has need at one time or another; or even that it should form all the ferments within its power each time that there is need for any one of them. The work of Jones[11] substantiates this view by showing that B. proteus will not form gelatinases on gelatin when any glucose is present; but that as soon as the glucose has been used up the gelatinases appear. This would indicate that the ferments are not preformed in the bacterial cell, but that they are elaborated in response to need. Such a concept would further suggest that the many different kinds of nitrogenous mediums would probably stimulate the production of more than one type of enzyme. For example, it does not seem probable that an amino-acid and a highly complex protein would call forth the production of identical ferments. The present investigation was undertaken to determine, if possible, the extent of the influence of mediums on ferment production.

## TECHNIC

The cultures to be studied for proteolytic enzymes were incubated at 37 C. for a period of 8 days. At the end of this time they were filtered through a sterile Berkefeld filter and 0.5 c c of the filtrate was added to sterile gelatin and sterile casein solution. Control tubes were made in each case for comparison; and tests as to the sterility always made, all filtrates showing contamination being discarded.

The gelatin mediums used to test for proteolysis contained meat-infusion broth and 15% of gelatin. To several tubes of this in the hardened form was added a portion of each filtrate and the tubes were placed in an incubator at 20 C. Several other tubes of gelatin were liquefied before the filtrate was added and then placed in an incubator at 37 C. Liquefaction in the latter case was determined by placing the tubes in ice water until the controls hardened. The results obtained by both methods coincided in every case, although the action was more rapid at the higher temperature.

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[11] Jour. Infect. Dis., 1916, 19, p. 33.

The casein solution was made up according to the method of Füld and Gross.[12] Ten gm. of Merck casein were dissolved in 100 c c of 10% $Na_2CO_3$ solution, enough 10% HCl added to just neutralize it and the volume made up to 500 c c with physiologic salt solution. The whole was then filtered, and diluted with physiologic salt solution until the medium showed a definite clouding on the addition of acetic acid, but no heavy precpitate. This was placed in small test tubes, 5 c c to a tube, and sterilized. To test for enzymes 0.5 c c of the filtrate under examination was added to each of several tubes and incubated for 24 hours, after which undigested casein was determined by the addition of 0.2 c c of a solution of 45 parts of 90% alcohol, 5 parts of glacial acetic acid, and 50 parts of distilled water. The absence of any precipitate indicated that all the casein had been digested. With each tube of casein a control was run for comparison.

Various methods have been utilized for the detection of gelatinases in bacterial cultures. Of these, probably the most widely used is the carbol-gelatin method in which 0.5 c c of the culture to be tested is added to a tube of gelatin containing 5% carbolic acid. This is supposed to prevent the growth of the bacteria but to have no influence on the action of the enzymes. Eijkmann[13] separates the enzymes from the bacteria by dialysis through agar, but the method seems impracticable when carrying out a large number of tests. Another method widely used is the one which gave the best results in our hands, namely, the separation of the enzymes from the bacteria by the filtration of the culture through a sterile Berkefeld filter. The method is laborious but, when properly carried out, very satisfactory.

Concerning the time at which the proteolytic activity of a culture is at its maximum, different views can be found. Von Gröer[14] states that the most gelatinases of B. prodigiosus are found in a culture 3 weeks old. Malfitano and Strada[15] found that on agar slants the proteases increase up to 24 hours and then decline. Mesernitsky[16] agrees with the work of Von Gröer on B. prodigiosus in finding that the gelatinases appear at the beginning of the 6th day and increase up to the 21st day. Bertiau[17] working with B. pyocyaneus and B. subtilis concluded that notable ferments exist in broth cultures at the end of 2 days and increase up to the 8th day, after which they decrease slowly. In our work all cultures were tested at the end of 8 days and, in case of negative results, were tested again after a greater length of time, but never did positive results appear in cultures which were negative at the 8th day.

## EXPERIMENTS AND RESULTS

The content of the mediums on which the organisms were grown was varied in order to determine what effect this would have on enzyme production. Gelatin itself was first tried and proteolytic enzymes were found to be produced by each of the organisms used. These enzymes, however, were not specific gelatinases because, although they liquefied gelatin rapidly, they also promptly digested the casein. This agrees with the findings of De Waele and Vandevelde[6] and others, and is the basis for some of the conclusions as to the identity of gelatinases and caseases.

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[12] Cited by Marras (Centralbl. f. Bakteriol., 1, O., 1914, 74, p. 505).

[13] Centralbl. f. Bakteriol., 1, O., 1904, 35, p. 1.

[14] Biochem. Ztschr., 1912, 38, p. 252.

[15] Compt. rend. Soc. de biol., 1905, 59, pp. 120, 195 and 197.

[16] Biochem. Ztschr., 1910, 29, p. 104.

[17] Centralbl. f. Bakteriol., 1, O., 1914, 74, p. 374.

When the bacteria were grown on the casein solution, proteases were again obtained which attacked both gelatin and casein and, with the qualitative methods employed, no difference between the activity of these ferments and the ones obtained from the gelatin mediums was apparent. However, later experiments lead us to conclude that with more accurate methods for the quantitative estimation of proteolysis some differences will very probably be found. The filtrates from cultures on meat-extract and meat-infusion both likewise were found to contain ferments which would rapidly digest both gelatin and casein.

After obtaining apparently identical ferments from these several mediums, all of which contained numerous complex proteins, the other extreme was resorted to, that is, a medium was utilized which contained no organic nitrogen. It is one which Magoon[18] recommends for the cultivation of tubercle bacilli and on which all of the common bacteria will usually grow well. It is made up as follows:

| | |
|---|---|
| Ammonium phosphate, dibasic | 1.75 gm. |
| Potassium phosphate, dibasic | 0.25 gm. |
| Sodium phosphate, dibasic | 0.50 gm. |
| Magnesium sulphate | 0.50 gm. |
| Glycerol | 20.00 c c. |
| Distilled water qs. ad | 1,000.00 c c. |

When all of the salts are dissolved, this is sterilized by fractional sterilization, after which it may be kept indefinitely and used as desired. Although this medium, due to the presence of the glycerin, is not strictly inorganic it will be called an "inorganic medium" in this paper for the sake of brevity.

When inoculated into this medium, which had been placed in small flasks, 100 c c to a flask, the organisms all developed rapidly and within a few days a heavy growth was obtained. At the end of 8 days a portion from each flask was filtered and the sterile filtrate added to gelatin and to casein solution as described. But on incubation no digestion occurred in any of the tubes. Other portions of the cultures were filtered and tested at 14 and 21 days, but in no case were any enzymes obtained which would attack either the gelatin or the casein.

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[18] Washington Agricultural Experiment Station, Bull. 132.

By means of the next experiments it was hoped to determine how close to gelatin and casein in chemical composition the mediums would have to be to stimulate the production of gelatinases and caseases. With this object in view peptone was added to the above described medium in a quantity sufficient to make a 1% solution. On this medium the organisms all grew vigorously, and it was found that they produced enzymes which would digest both gelatin and casein.

This showed the necessity for utilizing some substances still more simple as sources of organic nitrogen and, as asparagin is frequently used in nonprotein mediums, it was next tried, enough of it being added to the original medium to make a 1% solution. On this the bacteria showed rapid development and in the filtrates from the cultures enzymes were found to be present which both liquefied the gelatin and digested the casein, and with apparently equal rapidity.

These results led us to study the literature on the chemical composition of gelatin and casein by Fischer, Abderhalden and others. According to Matthews,[19] in addition to various percentages of most of the amino-acids, both of the above proteins contain aspartic acid, gelatin 0.56% and casein 1.4%. Gelatin also contains 16.5% glycocoll and casein none, while casein contains 4.5% tyrosin and 1.5% tryptophan and gelatin contains neither. Of these amino-acids tryptophan was not available, but glycocoll and tyrosin were utilized for our experiments, 1% solutions of each being made up with the "inorganic medium" as a base.

In the mediums containing glycocoll each of the 3 organisms were allowed to develop as in previous experiments but growth was found to be more slow. However, after several days a vigorous growth appeared in each of the flasks, and at the end of 8 days the cultures were filtered and portions of the filtrates tested for enzymes in the manner already described. Each one of these filtrates from the mediums containing glycocoll showed marked liquefaction of gelatin, but had no action on the casein. The rate of the liquefaction of the gelatin was also marked, being noticeably more rapid than that caused by the filtrates from any of the mediums previously used, even from gelatin itself. But on the casein solution there was no action, even when allowed to remain in the incubator for several days. Other samples from these cultures were filtered and tested at various intervals up to 3 weeks, but the results obtained were always the same.

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[19] Physiological Chemistry, 1916.

On the mediums containing the 1% of tyrosin growth was more rapid but the filtrates less active. In the tubes of gelatin, which were inoculated with filtrates from this medium, there was absolutely no evidence of liquefaction, either at 20 C. or at 37 C.; but in the casein solutions there was quite marked digestion by the filtrates from the cultures of each of the organisms. This digestion, altho not so rapid as that caused by trypsin, was apparently of about the same intensity as that obtained with the filtrates from broth, gelatin, peptone and asparagin.

In tabular form these results appear as follows:

TABLE 1

RESULTS OF EXPERIMENTS WITH VARIOUS MEDIUMS TO DETERMINE ENZYME PRODUCTION

| Source of Enzymes | Liquefaction of Gelatin | Digestion of Casein |
|---|---|---|
| Gelatin: | | |
| B. pyocyaneus | + | + |
| B. prodigiosus | + | + |
| B. subtilis | + | + |
| Broth: | | |
| B. pyocyaneus | + | + |
| B. prodigiosus | + | + |
| B. subtilis | + | + |
| Agar slant: | | |
| B. pyocyaneus | + | + |
| B. prodigiosus | + | + |
| B. subtilis | + | + |
| "Inorganic mediums": | | |
| B. pyocyaneus | 0 | 0 |
| B. prodigiosus | 0 | 0 |
| B. subtilis | 0 | 0 |
| 1% peptone: | | |
| B. pyocyaneus | + | + |
| B. prodigiosus | + | + |
| B. subtilis | + | + |
| 1% asparagin: | | |
| B. pyocyaneus | + | + |
| B. prodigiosus | + | + |
| B. subtilis | + | + |
| 1% glycocoll: | | |
| B. pyocyaneus | + | 0 |
| B. prodigiosus | + | 0 |
| B. subtilis | + | 0 |
| 1% tyrosin: | | |
| B. pyocyaneus | 0 | + |
| B. prodigiosus | 0 | + |
| B. subtilis | 0 | + |

## DISCUSSION OF RESULTS

The fact that nonspecific proteases can be obtained from cultures on gelatin, casein and nutrient broth has frequently been mentioned and is of no special significance in this paper, except to show the basis for most of the conclusions as to the identity of proteolytic enzymes.

These conclusions seem perfectly logical in view of such results, and the ones reached by us would have been very similar, had not experiments with other mediums thrown further light on the subject.

The finding of no gelatinases or caseases on mediums containing no organic nitrogen at first appears contradictory to the findings of some other writers, notably Jordan,[20] Aubel and Colin,[21] and Abbott and Gildersleeve.[22] But, on close investigation of their work, one finds that the "nonprotein" mediums of which they write always contain organic nitrogen in one form or another. Jordan, for example, in his experiments varies the salt content of the mediums, but in each case asparagin is found to be present. Aubel and Colin use asparagin or ammonium aspartate as the source of nitrogen. With such findings we can well agree, but on mediums containing no organic nitrogen we are forced to conclude that enzymes are not liberated by the bacteria. These results seem significant in the study of enzyme formation and furnish further evidence that bacterial enzymes are not preformed in the cell but are manufactured by the organism as the need for them arises.

The formation of both gelatinases and caseases on the mediums containing 1% of peptone is not surprising and has very little significance because peptone is a substance of rather indefinite chemical composition, which is found as an intermediate step in the breaking down of all proteins. For this reason, if it stimulated the production of any proteases at all, these would be expected to attack one protein as well as any other.

With asparagin as a source of nitrogen the production of gelatinases and caseases at first seems surprising and appears as another argument against the specificity of proteases. It is one which has been mentioned in the literature and would be a strong one were it not for the fact that asparagin is an amin of asparatic acid which is present in both gelatin and casein. Thus, proteolytic enzymes formed on mediums containing asparagin as a source of nitrogen might be expected to attack casein as well as gelatin. In regard to enzyme production in mediums containing inorganic salts plus asparagin, Jordan[20] states that the enzymes are produced in just as large amounts as they are in gelatin or in broth, but that a longer time is necessary. Our results, although not quantitative, seem to agree with this.

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[20] Biol. studies of pupils of W. T. Sedgwick, Boston, 1906, p. 124.

[21] Compt. rend Soc. de biol., 1913, 74, p. 790.

[22] Jour. Med. Research, 1903, 10, p. 42.

As to the nature of this enzyme our work merely shows that it is proteolytic, digesting both gelatin and casein. Effront and Ehrlich,[23] however, inoculated solutions of asparagin with yeast and obtained ammoniacal nitrogen. The filtrates of such cultures also had the property of breaking down asparagin and the amount transformed was proportionate to the time. This signified to them that amidase is formed which is responsible for the action. On the other hand, Walker and Kendall,[24] after studying the proteolytic enzymes of B. proteus, conclude that the one which liquefies gelatin is in a sense at least a preparatory one, preparing the protein for assimilation and that "deaminization is an independent phenomenon, associated with the intracellular utilization of the products of proteolysis." This work makes it appear as though there must be two enzymes formed on asparagin. but it is the proteolytic enzyme rather than the deaminizing one with which we are interested.

On glycocoll we found growth to be less vigorous than on the other mediums. This agrees with the observations of Bielecki[25] who found that bacteria will grow better on asparagin than on any of the other amino-acids which he investigated, but that some development is obtained on all. Franzen and Egger[26] also found that of asparagin, alanin, and glycocoll, asparagin forms the best source of nitrogen for B. prodigiosus, while the least growth is obtained on glycocoll. The enzymes obtained from this medium were very marked in their action, the gelatin being liquefied with surprising rapidity and the casein remaining unchanged.

On the mediums containing tyrosin the results were just as striking, the gelatin showing absolutely no liquefaction while the casein was progressively digested. The results seem quite significant in view of the fact that gelatin contains glycocoll but no tyrosin, while casein contains tyrosin but no glycocoll.

The ultilization in a similar manner of other amino-acids, particularly tryptophan, would doubtless further demonstrate this point but, due to the shortage of chemicals in consequence of the present war, it has been impossible to obtain them for this piece of work. However, the ones which were used point very strongly toward the conclusion that the amino-acids play a very important part in the determination of specificity among proteolytic enzymes.

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[23] Compt. rend. Acad. de sc., 1907, 146, p. 779.

[24] Jour. Infect. Dis., 1915, 17, p. 442.

[25] Compt. rend Soc. de biol., 1911, 70, p. 100.

[26] Centralbl. f. Bakteriol., 1, Ref., 1915, 63, p. 49.

It is as yet hard to say just how comprehensive the conclusions should be which follow from such findings; but it appears that, on mediums containing no organic nitrogen, no proteolytic enzymes are formed, and that, on mediums containing but one amino-acid as a source of nitrogen, the enzymes which are formed will attack only proteins which contain that particular amino-acid. In other words, this enzyme seems specific for proteins containing that one amino-acid. Thus, the enzymes formed on most amino-acids tend to show very little specificity because the great majority of amino-acids, like aspartic acid, are present in practically all of the proteins. Also, the ferments, which are formed on mediums containing either many amino-acids or a few more complex nitrogenous compounds, would have the ability to attack large numbers of proteins, thus appearing to be entirely without specificity.

Experiments similar to the ones described in the foregoing are apparently unmentioned in the literature. Consequently the results may offer a new foundation on which to base our ideas as to the specificity of bacterial proteolytic enzymes. That these enzymes are not specific for individual proteins has been the opinion of many reliable workers and yet the conclusion that one and the same enzyme breaks down all proteins does not follow from their work. They also state a priori that it is unreasonable to assume for each protein and protein derivative a special enzyme. The results of our experiments do not contradict this. They indicate rather that the number of ferments which a bacterial cell can liberate is limited and depends on the amino-acids present.

As to whether the amino-acids in the free and in the combined state exert the same influence on enzyme production has not yet been established. The results obtained by growing the bacteria on the usual protein mediums do not lead to definite conclusions concerning this point because one cannot be certain that these proteins do not contain some free amino-acids, particularly after having been exposed to the high temperatures necessary for sterilization. Some experiments at this laboratory to determine this point are as yet unfinished, but it is hoped that they may eventually throw some light on the subject. The method employed is as follows: Sterile gelatin is subjected under sterile conditions to dialysis for a time sufficient to remove from it all crystalloid substances. Then sterile salt solution is added to give the desired salt concentration and it is inoculated with bacteria which have first been transplanted several times on the mediums containing no organic nitrogen. The object of these transplants is to obviate the possibility of carrying any amino-acids or ferments onto the gelatin along with the bacteria. As can easily be seen, the difficulties in carrying out this technic are great and as yet no satisfactory results have been obtained. However, by a technic such as this we hope to procure some further definite information as to whether pure proteins stimulate the production of enzymes. This would confirm the work of Sperry and Rettger,[27] who found that pure egg albumin, serum albumin, and edestin were unattacked by a variety of proteolytic bacteria in the absence of other organic sources of nitrogen.

But, whatever the results of this experiment may prove to be, the conclusions from those already completed will be unaffected, namely, that the amino-acids are important factors in the determination of the nature of the enzymes which are formed, and that these enzymes are probably not specific for the proteins but rather for the amino-acids which go to make them up.

In regard to the enzymes formed on complex proteins several possibilities suggest themselves: (1) that only one enzyme is formed, and that this is modified by the presence of the various amino-acids so as to attack them all, or (2) that a separate enzyme is formed to correspond to each amino-acid present, and that these different enzymes are present approximately in the same proportion as the amino-acids which stimulated their formation. From our work the latter possibility seems the more probable, although none of the results show this conclusively.

With such results as a basis, we may be able to modify somewhat our ideas of the formation and the action of bacterial proteolytic enzymes. First of all, the enzymes are dependent on some inherent property of the organisms themselves. This is evident from the fact that some bacteria do not have the ability to form certain ferments, no matter on what mediums or under what conditions they are cultivated. For example, B. typhosus and B. dysenteriae, etc., have never been known to form gelatinolytic nor caseinolytic enzymes, and, although B. coli produces an enzyme which will form indol from peptone, it liberates none which will attack gelatin.

A second factor, or group of factors, which influences enzyme formation may be called general conditions. This includes the temperature, the reaction of the medium, the time of growth, etc., all of which have a greater or less effect on the production of enzymes by bacteria, and on which much exhaustive work has been done by such men as Kendall, Von Gröer, Jordan, Abbott and Gildersleeve, Bertiau, Emmerick, Fermi, etc. Their work shows that in the formation of enzymes there is for each organism a maximum, minimum and optimum temperature, and that in most cases the optimum is about 37 C. The optimum chemical reaction is also variable, a very slightly alkaline medium seeming best for the production of most enzymes. However, as they will act nearly as well in a neutral or slightly acid medium, this point seems to be of no great importance. The quantity of enzymes present in cultures of different ages has been discussed earlier in this paper. All of these observations, although of importance in the study of enzymes, show differences of quality only and so are not particularly pertinent to this question, for which reason they will not be discussed in greater detail.

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[27] Jour. Biol. Chem., 1915, 20, p. 445.

A third and most important factor in determining the character of the enzymes produced is the composition of the medium on which they are formed. This is demonstrated by the experiments reviewed in this paper, the results of which may be put in general terms as follows: If a protein medium is inoculated with an organism which has the ability to form ferments to correspond to a large number of amino-acids, enzymes which will attack practically any protein can be obtained from the culture. This may be due to the fact that, apparently at least, ferments are formed to correspond to all of the amino-acids present, and that these ferments then attack the acids in whatever form they find them. On mediums containing less complex nitrogenous substances or only amino-acids the same bacteria will give off ferments to correspond only to the amino-acids present, and these ferments will attack only substances containing these same acids. The vigor with which they will attack these substances seems to depend on the relative amino-acid content of the mediums on which they are formed and that on which they act, namely, if the medium on which the enzymes are formed contains only asparagin and glycocoll and the medium on which they are inoculated contains only a small percentage of one or the other, the digestive action will be much less marked than it would be if the protein medium contained more of one or both of the same acids. That is, the amino-acid content of the medium determines the specificity of the enzymes formed.

On the basis of these findings it seems that many of the so-called "irregularities of bacterial action" may be explained. Every bacteriologist finds organisms which, although corresponding according to morphology and pathogenicity to known types, show some "irregularity" in their action on culture mediums. Later, on a different lot of mediums or possibly at a different laboratory, the same organism may show the action on culture mediums which was previously expected, and the worker, at a loss for an explanation, concludes that it is merely another example of the "irregularity of bacterial action." Occasionally there may be some truth to this conclusion, but probably very often this "irregularity" would never have appeared if the chemical composition of mediums were always the same.

These same observations of the effect of mediums on bacterial ferments will probably furnish an explanation for many of the "new strains" of bacteria which are described, but are never verified by other workers. They usually differ from well established strains only in some minor points, and slight differences in the chemical composition of the mediums on which they are grown could well account for these.

Another phenomenon for which this conception of ferment activity seems to offer an explanation is the apparent incongruity of the action of some bacteria on mediums. For example, B. proteus and B. subtilis are well known to be strongly proteolytic and exhibit typical proteolytic actions, such as the liquefaction of gelatin and the formation of indol. On the other hand, B. coli and some of the dysentery group, although without action on gelatin, still form indol in large amounts from peptone. These organisms are not considered proteolytic and yet they unquestionably exhibit proteolytic activity when they split off tryptophan from peptone and then break down the tryptophan itself to form indol. If we accept the theory that all proteolytic enzymes are the same, we must expect these organisms which show proteolysis on peptone to liquefy gelatin. On the other hand, if we consider this in view of the findings given in this paper, the action does not appear at all surprising. Here are several organisms which have the inherent ability to form enzymes which will attack only one amino-acid and that is tryptophan. And in peptone we have a protein derivative which contains tryptophan, while gelatin is a protein in which no tryptophan is present. The explanation is self evident on this basis.

## CONCLUSIONS

On a medium containing no organic nitrogen, no proteolytic enzymes are formed by bacteria.

On gelatin, casein, broth, agar, and peptone enzymes are formed which will digest both gelatin and casein.

The ferments obtained from mediums containing amino-acids as the only source of organic nitrogen show various actions. The ones from asparagin digest both gelatin and casein; on glycocoll, no caseases are liberated; and on tyrosin, no gelatinases.

Proteolytic enzymes are apparently formed to correspond to the different amino-acids present in the medium and will then attack these acids whether combined or free.

These enzymes are not preformed in the bacterial cell but are dependent on the content of the medium on which it grows.

The specificity of proteolytic enzymes is resident in the amino-acids composing the proteins and not in the proteins themselves.

Differences in the chemical constituents of mediums may account for many of the unverified "new strains" of bacteria, the differentiation of which is based on some minor phase of their action on culture mediums.

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# THE PATHOGENICITY OF MOUTH STREPTOCOCCI AND THEIR ROLE IN THE ETIOLOGY OF DENTAL DISEASES

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# THE PATHOGENICITY OF MOUTH STREPTOCOCCI AND THEIR ROLE IN THE ETIOLOGY OF DENTAL DISEASES.

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OUR knowledge of mouth bacteria and their relationships to pathological conditions in and about the teeth is in a somewhat chaotic condition, and, at the present time, when interest in this branch of knowledge is undergoing a revival, it is perhaps wise to stop a moment and sum up what has already been established. The pioneer work of Miller has not been followed by productive investigations comparable to those made in other fields of bacteriology. There are several reasons for this. In the first place, the field has not promised to yield immediate tangible results of practical value, and during the youth of bacteriology as a science its investigators have largely been led to more fertile fields. Therefore, a considerable amount of the work published has been carried on by dentists, inspired by considerable enthusiasm for the work but frequently lacking the training necessary for conducting accurately investigations in so highly specialized a science. For this reason many of the micro-organisms described as new varieties associated with dental disease may probably be contaminating bacteria falsely identified. A bacteriological judgment must be developed before such work can be intelligently interpreted. In the second place, the flora of the mouth is so vast both in numbers and species, and so hard to cultivate, that the technical difficulties have been almost insurmountable. Thus Küster states that "All of the authors who have sought to clear up the bacteriology of the mouth by classifying every pure culture grown from the mouth were soon at the end of their ability. * * * Miller alone cultivated over a hundred different kinds of bacteria in a few weeks." Such attempts at classification have seldom served any useful purpose. On account of the failure of subsequent observers to differentiate accurately between contaminating germs and those actually present in the mouth, or between accidental visitants and normal inhabitants of the mouth; on account of their failure to determine accurately the biological characters or biochemical activities of the various mouth bacteria, or their pathogenic potentialities, Miller's vast collection of names and descriptions has remained to us an almost useless inheritance.

From time to time there have been described micro-organisms for which their discoverers have claimed a specific relationship to one or another dental disease. None of these, however, has stood the test of subsequent investigations and we are forced to believe, until stronger proof is forthcoming, that in dental infections we have to deal only with those organisms normally present in the healthy mouth. This conclusion has a corollary, namely, that the mere presence of bacteria will not cause dental infections, or we would all suffer from such diseases. Koch's postulates cannot be fulfilled. We may determine in a general way, by animal inoculation, the pathogenicity of the various germs encountered, but we cannot reproduce, with the bacteria alone, the same dental lesions with which they are associated in human mouth. This has led a number of dentists to view with skepticism the etiological relationships of bacteria and to seek the causes of dental disease elsewhere, as in disturbed metabolism, etc. While it is probably true that mouth infections require in their etiology some other factor, be it constitutional disturbance (such as diabetes and scurvy), or a local condition (such as accumulated tartar or an ill fitting denture;) yet a careful examination of the changes wrought in the damaged tissues leads one inevitably to the conclusion that these changes are the work of bacteria.

While a great many varieties of micro-organisms have been recorded as occurring in the mouth, there are only a few which have been found so constantly and in sufficient numbers that they can be considered characteristic of this region. They have therefore been the object of more intensive study. Fortunately, within recent years bacteriologists have largely abandoned the thankless task of classifying mouth bacteria and have devoted themselves to studying the varying flora in different parts of the mouth at different ages, the influence of varying conditions of cleanliness and health upon the mouth flora, and particularly to learn the disease-producing powers of the various mouth bacteria. Bearing in mind that practically all of the dental diseases are the result of the invasion of the teeth and their investing tissues by micro-organisms, we propose here to review some of these studies and to sum up the facts which we believe have been established.

The distribution of bacteria in various parts of the mouth, and the changes in the bacterial flora at different ages have been the object of a number of studies. These have been reviewed and considerably added to Madame Brãilovsky-Lounkevitch in a paper entitled, "Contribution a l'etude d la Flore Microbienne Habituelle de la Bouche Normale" (Annales de l'Institut Pasteur, August, 1915, xxix, p. 379.) This author has examined smears and cultures from tongue, cheeks, and gums of a number of individuals varying from new born infants to adults. As in all such studies, a large number of adventitious or accidental microbes have been encountered; thus the **Pneumococcus** was frequently encountered in the mouths of infants in hospitals, but was usually absent in children at home. These organisms, not essentially oral in their habitat, have been recognized and noted by the author as accidental visitants.

Her investigations have established certain important facts. The mouth at birth is sterile. From the 6th to 10th hours it begins to have a bacterial flora, recognizable at the 10th hour by direct microscopic examination. The first organisms found are various cocci (staphylococci, streptococci, the pigment-forming cocci of the air) and spore-bearing air bacteria. **From the first week until the appearance of dentition there is but one organism which may be considered constant, the Streptococcus salivarius. From the twelfth day it is the predominant species.**

With the appearance of teeth, the flora which was previously uniform thruout the mouth, changes; there are added certain other species which live by preference in the gingival crevice and interproximal spaces. These are the leptothrix threads, the spirochaetes, and certain anaerobic bacteria. They are constant and characteristic mouth bacteria, but their presence is to be explained not by any change in the constitution of the tissues of the mouth, but in the formation of spaces where food material may accumulate and putrefaction may occur; they are putrefactive bacteria. The flora of the adult mouth is practically the same as that of the infant after dentition. The **Streptococcus salivarius,** occurring as diplococci or short chains on the squamous epithelial cells, still forms the predominant species; on the cheeks it is practically the only organism, but on the tongue may be accompanied by some of the rods and spiral forms noted on the gums.

Recognizing the fallacy of the older investigations of the bacteriology of the mouth, Kligler has studied quantitatively, and apparently with some accuracy, the bacteria occurring on tooth surfaces and in cavities. By an ingenious technic the material removed was weighed and properly diluted. Counts were made both by direct observation thru the microscope and by plating on various media. In this manner, not only was the actual number of organisms present determined, but the infrequent and accidental forms were excluded, and the relative numbers of the different kinds of bacteria were made known. His studies yielded some very interesting results. The cases studied were divided into nine groups according to the condition of the mouth, starting with "Immunes" and passing thru increasing degrees of mouth dirtiness to advanced stages of caries. There was found, naturally, a progressive increase in the number of bacteria present from the first to the last groups. More interesting, however, were the findings in regard to the relative numbers of the various groups of organisms present. The streptococci, which form almost one-half of the organisms on the tooth surface in the healthy immune mouth, gradually decrease, and in caries are largely replaced by other forms, particularly the rods. It is to be pointed out that this decrease is only relative, and that if Kligler's percentages be converted into actual numbers there is found a very considerable increase of streptococci in the dirty and carious mouth. It is to be noted also, from his tables that, while the actual numbers of organisms in the dirty mouth is much greater than in the clean, the relative proportions of the various groups remains much the same. Caries alone, apparently, disturbs this relationship.

From a superficial examination of Kligler's data we are persuaded to believe streptococci are a negligible factor in caries, since they are relatively replaced on the decayed tooth surface by other bacteria, particularly acid-producing bacilli. Such, in fact, are his conclusions. Nevertheless, after a careful consideration of the data at hand, we are unable to concur in this opinion. We see in Kligler's figures merely a confirmation of the facts brought forth by Bräilovsky-Lounkevitch, namely, that when conditions of uncleanliness, and especially of decay, are present in the mouth, saprophytic bacteria tend to replace the purely parasitic mouth streptococcus, capable of living on the clean, healthy mucous membrane. It may be that dead dentine forms the pabulum of choice for certain saprophytic mouth bacteria. Kligler also offers as evidence to support his view, the high acid-producing power of the bacilli isolated. But it is to be pointed out that mouth streptococci themselves are no mean competitors in this regard. Moreover, acid-production alone is not sufficient evidence that an organism is the cause of caries. It may well be that the final dissolution of dentine is due to acid-production by certain bacilli, but it must also be remembered that dentine is, while relatively inert, nevertheless a living tissue; it has its organic matrix in vital relation with the odontoblasts and nourished from the pulp. Being a vital tissue it is more or less resistant to infection; no satisfactory chemical or physical theory has as yet explained immunity to caries. And any organism, to be accepted as the cause of caries, must exhibit an ability to invade and overcome living tissues, qualities which, as we shall see later, are possessed, par excellence, by the streptococci.

We have already mentioned that there occur in dental infections only a few varieties of organisms which are present in sufficient numbers or with such constancy that they may be considered as possible etiological factors in these diseases. We propose next to consider individually these various organisms and to review the more important papers bearing upon their virulence, since a study of the pathogenic properties of the bacteria concerned is of prime importance in establishing their relationship to mouth infections. This property of virulence is itself made up of two more or less interdependent factors, the ability of the organisms to invade living tissues, and their power of forming toxines. Since none of the mouth bacteria concerned in dental infections produce, so far as is known, soluble toxines or very potent endotoxines, we may confine our observations to the invasive powers of the micro-organisms. Bacteria may invade the tissues locally (microscopic and cultural studies of their presence in the deeper parts of the teeth and their investing tissues) or may invade the blood and lymph channels and produce lesions in distant parts (metastatic infections). We may also demonstrate the invasive powers of organisms by their ability to survive and multiply when experimentally inoculated into laboratory animals.

The organisms which we believe deserve consideration in this study are the fusiform bacilli, the mouth spirochaetes, the **Leptothrix buccalis**, the **Endamoeba buccalis**, the mouth staphylococci, and the streptococci. Of these, we hope to show that the last group alone, the strepococci, are the primary cause of the various dental infections; caries, pulpitis, alveolar abscess and pyorrhea.

**Fusiform Bacilli.** The spindle-shaped bacteria of the mouth first observed by Plaut and Vincent in a characteristic type of ulcerative tonsillitis have been considered by many to be specific organisms and the cause of that disease, commonly called Vincent's angina. It has since been shown, however, that they are normal inhabitants of the healthy mouth and that they may be associated with a wide variety of ulcerative and gangrenous processes in various parts of the body. They are, however, rather widely distributed in nature and have been found in many of the lower animals.

Morphologically, the organism is characteristic. Its tapering pointed ends, and the presence of certain peculiar granules, staining deeply with those dyes that have an affinity for chromatin, permit of its immediate identification under the microscope. Certain discrepancies in the reports of various observers on the reaction to Gram's stain and the motility of these organisms seem to indicate that several species have been included under the name **Bacillus fusiformis**, and our own observations of forms found in pyorrhea pus have convinced us that there may be found here two distinct species, both spindle-shaped and containing nuclear granules, but differing in the following features: one (and this is the rarer of the two) is somewhat thicker, is Gram negative, possesses active motility, and has numerous peritrichic flagella, while the other is more slender, Gram positive, motionless, and tends to gather in clumps.

The fusiform bacillus may be cultivated anaerobically in media containing blood serum. It requires considerable time and patience to obtain a pure culture. In culture media the morphology changes somewhat, the organisms tending to grow out in longer filaments, and in older cultures and upon certain media there is a tendency to produce bizarre involution forms, the rods twisting up into spiral forms. In cultures there is produced a most disagreeable odor of putrefaction which is characteristic and is identical with the odor in the mouth noted in ulcerative and gangrenous processes; the same odor is frequently noted in opening old alveolar abscesses and gangrenous pulps.

The fusiform bacilli are almost invariably associated with certain spirochaetes. They are found in the healthy mouth in relatively small numbers, in the gingival crevices and interproximal spaces, and in the tonsilar crypts. They are always present in increased numbers in pyorrhea pus. They are particularly associated, however, with ulcerative and gangrenous processes.

They have been found in large numbers in certain types of ulcerative stomatitis, particularly gingivitis, and in the characteristic form of ulcerative tonsillitis mentioned above. They form a large part of the mixed flora of noma, or gangrenous stomatitis. They were first observed by Vincent in hospital gangrene a type of gangrenous wound infection seldom seen since the advent of asepsis. They occur in the lesions of the feet and legs known as tropical ulcer. In all of these conditions, however, the organisms are found in the superficial portions: the surface exudate, the dead tissues, or, at most, the very superficial part of the active process in the living tissue. In this respect, i. e., their inability to invade the tissues, they resemble the diphtheria bacillus, but differ in that they form no demonstrable toxine that may be absorbed and destroyed tissues. They never occur in these lesions in pure form, but are invariably associated with cocci, with spirochaetes, and usually thread forms. Moreover, they have frequently been found associated with ulcerative and gangrenous processes in which the cause is known; thus, we find them multiplying in tissues rendered gangrenous by interference with circulation, or by frostbite, in the ulcers of syphilitic and mercurial stomatitis and diphtheria.

Metastatic infections with fusiform bacilli are exceedingly rare, and if we exclude those cases in which other bacteria occurred in the metastatic abscesses, we have but six cases to record. Ghon and Mucha reported two cases of brain abscess secondary to bronchiectasis and gangrenous appendicitis respectively, in both of which there were found fusiform bacilli and long thread-like forms. The latter resembled the involution forms of fusiform bacilli observed in cultures. Kasper and Kern saw a similar case in which the same forms were noted in multiple abscesses secondary to appendicitis. The long threads, in their case, grew in cultures to look like the normal fusiform bacillus. Rosenow and Tunnicliffe had a very similar case. Frühwald noted fusiform bacilli alone in a brain abscess following a wound of the pharynx. Larson and Barron obtained a pure culture of fusiform bacilli from the blood, from a patient dying of ulcerative stomatitis and gangrenous inflammation of the superior maxilla.

These few cases alone do not furnish any proof of the pathogenicity of the fusiform bacilli. In all but two of them there occurred long thread forms as well as the fusiform bacilli, which may or may not be involution forms. In all but one they occurred in patients weakened by the intoxication of an extensive gangrenous process. The other cases of metastatic infection in which fusiform bacilli were found have all shown other organisms as well, particularly pyogenic cocci, so that we cannot consider them as offering any evidence of the invasive properties of the bacilli. Most of them contained a great variety of organisms, indicating that the metastasis occurred by direct extension from one of the body surfaces, or that gross particles containing a number of bacteria were mechanically set free into the blood stream; they do not indicate any ability of the bacteria to invade the vascular channels. Such cases are those reported by Veszpermi, Costa, Silberschmidt, Feldman, Dick, and the second case of Kasper and Kern.

The direct inoculation of pure cultures of the **Bacillus fusiformis** into laboratory animals fails to produce any infection. It is true that Ellermann noted small local abscesses following subcutaneous inoculation into a guinea-pig, and Paul was able to produce a purulent peritonitis with one culture obtained from pyorrhea pus, but these observations stand alone in a large series of experiments carried on by many investigators in which sometimes very large doses were injected subcutaneously, intraperitoneally, intravenously, intraocularly, and intratesticularly into a variety of animals. The inoculation of pus from metastatic abscesses in which the fusiform bacillus alone was found has resulted negatively in those cases where such experiments were performed. On the other hand, the direct inoculation of pus, or material from the mouth, in which the organism occurred together with other bacteria, has yielded positive results in some cases. These experiments have been reported by Veszpremi, Silberschmidt, and Costa; the latter found that the fusiform bacilli survived in guinea-pigs after some weeks. Such experiments, however, cannot be offered as proof that the organisms are primarily virulent; they merely show that the fusiform bacillus can survive and multiply in pus and necrotic tissue produced by the pyogenic cocci in the original material.

Of dental lesions, the fusiform bacilli are found more constantly in pyorrhea than in other diseases. They prefer the gingival crevice, and are not numerous on tooth surfaces, even when the latter are filthy and loaded with bacteria. They are not prominent in caries and are not at all present, as far as we can learn, in the deep layers of carious dentine. They are frequently present in chronic alveolar abscesses, but are not constant. In pyorrhea pus, however, we have seldom found them absent, and they are usually present deep in the pockets. We have been unable to demonstrate their presence in the tissues in microscopic sections from pyorrhea.

Gins states that "The **Bacillus fusiformis**, as well as the spirochaetes, both apparently require a special preparation of the body tissues before they can multiply in them," and Ellermann arrives at similar conclusions; after noting that these organisms multiply to a considerable extent in diphtheria and other infections, the latter author states that the fusiform bacilli may in Vincent's angina and noma also live a purely saprophytic existence, acting as harmless commensals rather than active pathogenes. Aside from the very few cases of clear metastatic infection, there is no evidence that these bacteria are capable of producing disease. We are forced to conclude that they are mere camp followers, harmless putrefactive germs living on the tissues killed by other more virulent microörganisms. Therefore, they can play only a secondary role in the etiology of dental disease.

**Mouth spirochaetes.** Spiral organisms from the mouth are illustrated in our oldest bacteriological records (von Leeowenhoek) and have occupied a prominent position in the writings of all who have devoted themselves to this branch of bacteriology. We must differentiate the rigid bacterial forms moving by means of flagella (spirilla) from the flexible forms moving by vibrations of the entire cell or by means of undulating membranes, and generally classed with the protozoa (spirochaeta). To the former class belongs the **Spirillum sputigenum** of Miller, cultivated by him aerobically on gelatine. The latter class has only recently been cultivated and requires anaerobic conditions and special media.

The variations in size and the number, depth, and regularity of the spirals indicate that several morphologically distinct species of spirochaetes occur in the mouth, and a large number of names for various forms are recorded in the literature. Hartmann and Mühlens, and Hoffmann and von Prowazek, independently divided the mouth spirochaetes into three species with distinct morphological characters and have to a considerable extent removed the confusion surrounding this group of micro-organisms. They recognize a **Spirochaeta buccalis**, the largest form with a few broad, flat spirals; a **Spirochaeta dentium**, the smallest form, with more numerous, narrower, and regular curves; and a **Spirochaeta media** exhibiting morphological characters intermediate between the other two. **S. buccalis** stains reddish with Giemsa's stain, while **S. dentium** takes a bluish tinge. Mühlens was able to obtain a pure culture of the smallest form in horse serum agar. Noguchi obtained pure cultures of the **Spirochaeta media** and **Spirochaeta dentium** and established firmly their morphological differences; he gave them new names, **Treponema macrodentium and Treponema microdentium**, thus separating them altogether from the larger, as yet uncultivated form, **Spirochaeta buccalis.** Noguchi later cultivated, from pyorrhea, another spirochaete that produced mucin and a fetid odor in cultures, which he names **Treponema mucosum.**

Mouth spirochaetes are found in the same areas in the mouth as the fusiform bacilli, except that they multiply to a greater extent on tooth surfaces and in the necrotic material of cavities. They increase in numbers in similar pathological condition, i. e., Vincent's angina, ulcerative stomatitis and noma and are constantly associated with the spindle-shaped bacteria in hospital gangrene and tropical ulcer. This constant association has led certain investigators to suggest that the fusiform bacilli and spirochaetes merely represent different morphological stages of development of the same organism (Plaut, and Wright), and Tunnicliffe has described spiral forms, found in old cultures of fusiform bacilli, which she believed indicated a transmutation of the two organisms; similar spiral forms were observed by Larson and Barron. Shmamine, however, showed that these were merely involution forms of the fusiform bacilli, and had nothing in common with the spirochaetes, while the subsequent pure cultivation of the latter finally proved that the relationship between the two microbes is purely symbiotic. Moreover, the occurrence of either form with exclusion of the other has been reported in ulcerative stomatitis (Ellermann) and in tropical ulcer (de Meyier), while the occurrence of fusiform bacilli in pure culture without spirochaetes in metastatic abscesses gives the final proof that the organisms are distinct. Their almost constant occurrence, together is to be explained by their similar biological characters, requiring anaerobic conditions and native serum for artificial cultivation, and increasing in the body in the presence of necrotic tissue.

Whether the spirochaetes associated with fusiform bacilli in ulcerative and gangrenous conditions are identical with those found in the normal mouth, and if so to which species they belong, is not altogether certain, but the balance of evidence seems to indicate that the larger **Spirochaeta buccalis** is most frequently found in this symbiotic relationship.

While spirochaetes have been found to advance slightly beyond the fusiform bacilli in the border area between the gangrenous and healthy tissues in noma (Ellermann, Buday), in gangrene of the lung (Buday) and in tropical ulcer (Keysselitz and Mayer), we have been unable to demonstrate their presence in a few specimens of tissues from pyorrhea stained by Levaditi's method, nor have we been able to obtain any other evidence of their pathogenicity. No metastatic infections have been reported in which mouth spirochaetes have occurred alone, altho they frequently accompanied the fusiform bacilli in those cases to which we have already referred, where a multiplicity of bacterial forms were found in abscesses secondary to gangrenous processes. Moreover, the mouth spirochaetes in pure culture have proven non-pathogenic for laboratory animals. Mühlens obtained negative results from his inoculation experiments. Noguchi found his **Treponema macrodentium** would produce a temporary induration in the testicles of rabbits and supra-orbital tissues of monkeys; the organisms, however, failed to multiply in the indurated tissues. With his **Treponema mucosum** there was produced a small amount of pus in the testicle of a rabbit, in which the spirochaetes survived, if some agar (foreign body) was simultaneously introduced into the tissues. Paul cultivated both fusiform bacilli and spirochaetes in pure form from pyorrhea pus, but found that the fusiform bacilli alone would produce infection in guinea pigs. It is also to be noted that in those cases where positive results were obtained from the direct inoculation of pus containing fusiform bacilli and spirochaetes in symbiosis, the former alone survived in the resulting lesions (Costa). We must therefore conclude that the mouth spirochaetes are without pathogenic properties.

**Leptothrix Buccalis.** Those organisms growing in long threads form so conspicuous and easily recognized a portion of the mouth flora that in the early articles on this subject they received a great deal of attention. The name has been applied to a large number of organisms, and the confusion in the literature is almost hopeless. By various writers practically all of the mouth bacteria have been considered at one time or another to be some stage in the development, or mutation form, of this micro-organism. Miller clearly recognized this confusion and the many errors in the work of his predecessors, and stated, "As absolutely nothing was known concerning the biology and pathogenesis of this organism, all sorts of wonderful properties were ascribed to it. It is therefore high time to banish this confusing name from bacteriological writings."

Miller's work was published in 1890, and yet, while the thread forms fail to attract attention today as factors in the production of disease, there remains almost the same confusion in classification and nomenclature. We are to differentiate those larger thread forms which belong to the higher fungi, or moulds, and which are occasionally found in the mouth. The true thread fungi, or trichomycetes, are very slender filaments without articulations, altho they may show irregularities in staining. These are subdivided into genera according to the presence or abscence of branching. Branched forms are given the name Streptothrix and such forms are at times found in the mouth and have been cultivated and named **Streptothrix buccalis** by Goadby; they have also been studied by Kligler. No distinguishing characters are given by these authors, however, as means by which they can be differentiated from the similar forms so constant in air, dust, and soil, and the mouth forms may be merely accidental visitors from the air, like **B. subtilis** and **B. mesentericus.**

The term leptothrix should be applied

only to the non-articulated unbranched threads, and until further accurate information concerning the differentiation of these forms is available, it will be well to retain the name **Leptothrix buccalis** for such non-branching thread forms as are found in the mouth, keeping in mind, however, that the name probably includes a group of organisms and not an individual species.

Leptothrix forms are found in intertwining masses on tooth surfaces and invade the area of dead dentine in caries. They are found usually also in the gingival crevice and in pyorrhea pus. They multiply most, however, in the materia alba on the surface of teeth, and their tendency to form adherent masses has been considered of importance in forming those plaques on the surface of enamel beneath which caries is supposed by some to develop; Klebs saw, in these plaques, the nidus about which salivary calculus was deposited. While in the older literature many local and general disease conditions were ascribed to this organism, it has not been shown to invade the tissues of the mouth except when such tissues are dead. Thus it is found in large numbers in noma, and was considered by Perthes to be an etiological factor in that disease; invasion of the living border of tissue has not, however, been demonstrated. While leptothrix forms have been found in some cases of endocarditis, in rat-bite fever, and in the urine in certain cases of kidney disease, identity of these organisms with the thread-bacteria of the mouth has not been established, and, save for the instances to be cited below, we can find no records of metastatic infection with **Leptothrix buccalis.** The organism has been grown in pure culture by Kligler, who reports no animal experiments. Wherry and Oliver also claim to have cultivated this organism, but likewise failed to inoculate animals. Thus it will be seen that the **Leptothrix buccalis** has not been proven to have any pathogenic properties.

There remains to describe a peculiar symbiotic relationship frequently entered into between certain fusiform bacilli and leptothrix threads. We frequently find on tooth surfaces and in pyorrhea pus, and occasionally in apical abscesses, small granules within the limits of visibility by the naked eye, which under the low power lens of the microscope are seen to be composed of radiating filaments with swollen extremities which bear a remarkable resemblance to the ray-fungi of actinomycosis. Such granules are also frequently found in the crypts of the tonsils. On finer examination, however, we note that these granules are composed of leptothrix threads to which there are mechanically adherent large masses of fusiform bacilli, especially at the extremity of the threads, thus producing the swollen "clubs" at the periphery of the granules. The structure of these granules has been demonstrated by von Beust, Veszpremi, and Davis, who have shown that these granules have nothing in common with **Actinomyces.** In the case reported by Veszpremi, these granules were found also in the brain abscess which developed by extension from the necrotic process in the jaw, and this is the only record we have of the occurrence of mouth leptothrix threads in metastatic infections, while here they did not occur alone.

The numerous reports in the literature of the occurrence of **Actinomyces,** without actinomycosis, in the crypts of the tonsils (Johne, Volkman, Wright, Lord) and in decayed teeth and alveolar abscesses (Volkman, Lord, Zilz, Partsch, and Thoma) lead one to believe that these granules have repeatedly been mistaken for the true ray fungus. This peculiar attachment of fusiform bacilli to leptothrix threads also probably formed the "fruitful heads" of the **Leptothrix Racemosus** of Vincentini to which he ascribed such wonderful properties.

**Endameba Buccalis.** An etiological relationship between this protozoan parasite and pyorrhea, suggested by Barrett and Smith, was more positively claimed by Bass and Johns. We have previously given, in the pages of this journal, reasons why we believe the mouth ameba are not pathogenic. Those reasons are, briefly: that the organism is present frequently in healthy mouths; that it does not invade the tissues; that its presence in pyorrhea pus in increased numbers can best be explained by the fact that here it obtains its favorite food, pus cells and certain bacteria; and, finally, that the administration of emetine, while it frequently destroys the endameba, fails to stop the disease process. These statements are still valid. We wish to emphasize that, unlike the endameba histolytica in dysentery, the mouth ameba cannot be found in sections of the tissues of pyorrhea, and have never been demonstrated in metastatic infections, altho Evans, Middleton, and Smith have suggested a possible relationship to thyroid disease.

Since the enthusiastic publications of Bass and Johns, there have appeared a number of other studies of the **Endameba buccalis**. Craig reviewed the morphology of the organism more thoroly than had been done before and described its life history; he failed to find any evidences of pathogenicity. Williams, Von Sholly, Rosenberg, and Mann, of the New York City Department of Health, examined a large number of children and found the ameba in 29% of cases with healthy gums, in 37% with healthy gums and decayed teeth, in 65% with tartar and receding gums, and in 91% with spongy, bleeding gums. These figures were closely paralleled by those of Mitchell. Culpepper, and Ayers at Syracuse. The latter observers found the ameba more numerous in superficial smears. Sanford found the organism present in 14% of health mouths; he pointed out, as did Craig, that there is no parallelism, either clinically or morphologically, between **Endameba buccalis** and **E. histolytica**, and stated, as did Craig also, that there is no proof that emetine always kills ameba in the mouth. Sanford failed in an attempt to transfer the ameba to lower animals.

Barrett and Smith claimed to have partially succeeded in cultivating the **Endameba buccalis**, but Craig pointed out that their "reproduction by budding" was an evidence of degeneration. Wherry also claimed to have grown the ameba on agar made from Martin's broth, but his evidence is not convincing. No animal experiments are reported, nor were pure cultures obtained. Hecker, by an ingenious modification of the Barber pipette succeeded in picking up a number of the ameba from pyorrhea pus, and after freeing them of bacteria with a finer pipette, introduced them into the gingival crevices of guinea pigs, and himself, without infection taking place.

We have, then, no trustworthy evidence that the mouth ameba are capable of producing disease; and this statement is equally applicable to the other large protozoa of the mouth, the more actively motile Endameba Kartulisi,, and the **Trichomonas buccalis** which has recently been studied morphologically and cultivated by Noguchi.

**Staphylococci.** The staphylococci of the mouth were first studied by Biondi. Injecting saliva subcutaneously into guinea pigs, this author frequently found that abscesses developed from which the **Staphylococcus pyogenes aureus** or **albus** could be cultivated; he also described another form which he named **Staphylococcus salivarius pyogenes,** differing from the others only in its small size. Staphylococci received but scant attention from the earlier writers on mouth bacteria. Black found the aureus variety seven times, and albus four times, out of ten healthy mouths. But Miller, Netter, and Vignal found them rarely. Goadby cultivated **S. p. aureus** 80 times from 1000 mouths, but found **S. p. albus** quite frequently, in the majority of the suppurative conditions of the mouth: He notes that in its slow rate of liquefaction of gelatine, and its low pathogenicity, the white staphylococcus of the mouth resembles the sub-variety known as Staphylococcus epidermidis albus. Kligler studied 29 strains of staphylococci, and found but two of the golden variety; of the twenty-seven white strains, twenty-one failed to liquify gelatine. Our own observations, in general, confirm those of Goadby and Kliger, that the **Staphylococcus aureus** is rare, and is to be considered accidental. The variety most commonly found is the non-liquefying white staphylococcus, generally considered non pathogenic.

The question of pathogenicity of mouth staphylococci needs considerable further study. As evidences of local invasive qualities, we note that they were found constantly in the deepest layers of carious dentine by Goadby and Kantorowicz. The latter author found **S. p. aureus** only once, and the majority of his **albus** strains were non-liquefiers. Staphylococci have been most frequently associated with the so-called suppurative processes, pyorrhea alveolaris and chronic alveolar abscess. Staphylococci, when they are pathogenic, are pus producers. But, as will be shown in a later paper, pyorrhea and chronic alveolar abscess are not primarily purulent infections at all; suppuration occurs as a secondary manifestation, and, in the case of the latter of the two diseases mentioned, is frequently lacking altogether. We have found staphylococci most frequently in pyorrhea, where suppuration occurs constantly; but in those cases where we have carefully cleansed and cauterized the pockets, and made our cultures from the deep tissues curetted out, we have frequently obtained pure cultures of the more contant streptococcus. We feel, therefore, that in pyorrhea the staphylococci are secondary and but superficial invaders, probably responsible for the suppuration. In chronic alveolar abscess, which is really a granulomatous process, we do not have the tissues exposed to the mouth cavity as in pyorrhea, and secondary suppuration occurs less frequently. We have not found staphylococci very often in apical lesions; unfortunately, we have not kept record of the number of times they have been found, but it is not more than ten per cent. Staphylococci occur usually where there is pus in the granuloma.

We have at hand no data concerning the occurrence of metastatic staphylococcus infections secondary to dental lesions. That they occur is granted; their rarity is indicated by the paucity of the literature on the subject. Animal inoculations have at times been successful. Biondi's results have been confirmed by others; that is, when saliva or mixed cultures from the mouth are injected into animals, abscesses containing staphylococci sometimes develop. We have found some of our strains pathogenic, but not constantly nor even frequently. Further study of this problem is indicated.

To sum up, the staphylococci of the mouth belong to the non-liquifying white variety generally considered non-pathogenic. They are found at times in dental and peridental infections, but not constantly, and show only a slight local invasive power; they are perhaps responsible for secondary suppuration in these lesions. Their pathogenicity is doubtful.

**Streptococci.** The earlier investigators of mouth bacteria were handicapped by the lack of modern methods of cultivating and identifying bacteria, particularly in that gelatine was used for isolating cultures, requiring incubation at room temperature. It is not surprising, then, that most of them failed to observe the most numerous, constant, and important group of mouth bacteria, the streptococci. Biondi, in his experiments with direct inoculation of saliva into animals, found a streptococcus which he considered identical with the variety that causes erysipelas and abscesses, which he named "**Streptococcus septo-pyaemicus.** Black described several varieties of streptococci in the mouth, "**Streptococcus pyogenes,**" for instance, in three out of ten samples. Netter obtained this organism in 5% of his cases, but Miller found it only once, and Vignal never.

Von Lingelsheim first offered a basis for classification of streptococci, and pointed out that the salivary streptococci differ from the forms found in abscesses and erysipelas, generally called **Streptococcus pyogenes,** in that they tend to form short chains, produce cloudiness in broth, grow on potato, and are non-pathogenic for mice. For these short-chained forms found in the mouth he proposed the name **"Streptococcus brevis,"** calling the pus-producing form **"Streptococcus longus."** It has since been shown repeatedly that the length of the chains is not constant nor a criterion of virulence, but in a general way von Lingelsheim's classification is correct, and he called attention to the large group of relatively non-pathogenic mouth bacteria. Goadby was struck by the **constant** presence of streptococci in the mouth. He states that "In all mouths, clean or dirty, I have never failed to obtain the Streptococcus." This fact, so easily demonstrated, is not as widely known as it should be, and is not readily accepted by those who have had little experience in studying the mouth flora. It should be pointed out that the finding of streptococci in cultures from any part of the mouth surface is without significance as far as disease is concerned, since such streptococci are **always** present in all parts of the mouth in enormous numbers, from shortly after birth thruout life. Goadby further described accurately the morphological and cultural characters of the mouth streptococci. He called attention to the fact that in the mouth they occur normally as diplococci. growing in pairs and not in chains. In this situation they are found adherent in large numbers to the squamous epithelial cells, or free on tooth surfaces. In culture media they tend to grow out into chains, usually short, but may remain as diplococci for some time; frequently they form chains of pairs. They show a marked tendency to become elongated in the axis of the chain. Goadby identified the mouth streptococci with the **"Streptococcus brevis"** of von Lingelsheim, also observing that it was not pathogenic for laboratory animals.

An approach to a more accurate differentiation of streptococci was begun by Gordon; in an attempt to establish the identity of mouth streptococci with those found in the air, he applied to this group of organisms the carbohydrate-fermentation tests that had proven so successful in differentiating the colon-typhoid bacilli. This work was continued and extended to other streptococci by himself, Houston, and Andrewes and Horder. The latter authors developed a working classification that is still acceptable. Among other forms they recognized a **Streptococcus salivarius,** characteristic of human mouth, which could be differentiated by its fermentation of certain sugars. Another step forward in the classification of streptococci was made by Schottmüller, who found that this group could be sharply divided into two by the use of blood agar; one sub-group dissolving the blood and producing a clear halo around the colonies, he called **"Streptococcus longus"** after von Lingelsheim, while the other sub-group was named, **"Streptococcus viridans"** since it failed to hemolyze, but produced a greenish zone.

We need not go further into the literature on the classification of streptococci; this has been dealt with more thoroly in previous papers from this laboratory. Suffice it to say that it was ultimately proven that the **streptococcus brevis** of von Lingelsheim, the **Streptococcus salivarius** of Andrewes and Horder, and the **Streptococcus viridans** of Schottmüller are one and the same organism, and are identical with the **"Diplococcus rheumaticus"** of Poynton and Paine. There has arisen some confusion in the literature due to the failure of various observers to differentiate accurately cultures from dental lesions, and we believe that it would be profitable to point out here the means by which the mouth streptococcus may be differentiated from other forms. The tendency to grow in pairs of somewhat elongated cocci has led to confusion with the Pneumococcus; it differs from the latter in its failure to produce capsules in inoculated animals, its failure to ferment inulin, and its insolubility in bile and bile salts. Differentiation from the more pathogenic **Streptococcus pyogenes** should be made by observation of blood agar cultures. While the mouth streptococci regularly fail to hemolyze blood and usually produce green colonies when the proper technic has been followed, it should be recognized that if very thin blood agar is used, or if the cultures be observed when several days old, the colonies will frequently be found to have a very narrow zone of hemolysis. We feel that this has been a source of error in the frequent reports of finding hemolytic streptococci in chronic alveolar abscess which we have been unable to confirm. We recommend, for sharp differentiation, the use of 20 drops of rabbit's blood in ten c. c. of agar and observation at the end of twenty-four hours. We also wish to call attention to the fact that non-hemolytic streptococci are found constantly in other parts of the body; that there occurs normally in the intestines a variety of **Streptococcus viridans,** called **Streptococcus fecalis** by Andrewes and Horder, which is differentiated from the mouth streptococcus by its ability to ferment mannite.

Are mouth streptococci a distinct species, or are they but a variation of the ordinary **streptococcus pyogenes**? Is there but one streptococcus which is highly variable, or are there numerous species? This is one of the most hotly debated topics in bacteriology, and we must admit is still unsettled. We believe that the balance of evidence at present is in favor of the view that there are a number of species of streptococci, distinct and not transmutable, of which the **Streptococcus salivarius** is one; certainly the division into hemolytic and non-hemolytic strains is, in our opinion, inviolable. The most radical opinion opposed to this theory is that of Rosenow, who believes that **Streptococcus viridans** may change into **Streptococcus pyogenes** on the one hand, and the **Pneumococcus** on the other. This theory has led to the wildest speculations as to the possible dangers of focal streptococcus infections. We wish to call attention to the fact that Rosenow's experiments have not, so far as we know, been confirmed by any other bacteriologist; that one author at least, Holman, has repeated those experiments without confirming them, and has pointed out the probable sources of error in Rosenow's work.

The pathogenicity of mouth streptococci is a very broad topic, and has been considered to some extent by us in previous papers. We wish to discuss here particularly the **primary** invasive qualities of the organism and its etiological role in the principal dental diseases. For this purpose we have gathered together several of the more recent papers on the bacteriology of caries, pulpitis, alveolar abscess, and pyorrhea. It would take us too far afield to review all of the literature bearing on these problems, and the older literature, particularly, is useless. As Kantorowicz stated in the introduction to his studies of caries, the older writers, (Miller, Vignal, Galippe, etc.) dealt only with the putrefactive bacteria living on the surface of the carious dentine, and failed altogether to observe the organisms characteristic of caries present in the depths of the diseased tooth substance. Moreover, using gelatine for isolation, they failed to get a growth of the more parasitic forms that grow only at body temperature. This criticism is equally applicable to the early studies of the other dental lesions, particularly pyorrhea. These papers, however, have greatly confused the study of dental bacteriology and have given rise to the erroneous impression that infections of the teeth and their investing tissues are always hopelessly mixed infections, and that since so many kinds of bacteria are present, no particular one can be considered the etiological agent. As a matter of fact, all of the later students who have attacked these problems rationally by developing a technic whereby surface organisms are excluded and only those bacteria deep in the advancing border of the diseased tissues are retained, have come to the same conclusion, namely, that the deeper you go into the tissues the more simple and uniform does the flora become, until ultimately streptococci alone remain.

This newer conception of dental bacteriology had its beginnings in the work of Sieberth on pulpitus. Previously it had been claimed by Arkövy that pulpitis was due to an organism named by him the **Bacillus gangrenae pulpae**, and the same organism was found by his pupil, Dobyzyniecki, in caries and alveolar abscess; but it was later shown by various workers that this organism was merely the common spore-bearing aerobe, **Bacillus mesentericus**, that had contaminated his cultures; this is but an example of a large number of studies of dental bacteriology whose authors lacked sufficient training in general bacteriology to interpret properly their findings. Sieberth chose teeth with dead pulps that were not sufficiently exposed by decay to be contaminated by the surface bacteria of the mouth, and, after cleansing the surface with disinfectants, cracked them open with sterile instruments. Cultures were taken from the pulp so exposed, and spread on agar plates. He relates his great astonishment at finding on these plates not the great variety of colonies of different kinds of organisms that he had been led to expect from the writings of previous workers, but only myriads of tiny gray colonies of streptococci. Only occasionally were other (contaminating) organisms encountered, and he justly concludes that streptococci alone are concerned in the devitalization of the pulp. Sieberth worked before the modern methods of differentiating streptococci had been developed, and altho he gave minute morphological and cultural descriptions and divided his strains into a number of groups, we are unable to determine whether or not they are the true salivary streptococci. Some strains were pathogenic for mice.

Goadby first separated the bacteria of caries into two groups, superficial and deep, and simplified the problem by showing that in the deep layers, only a few varieties of organisms were present. By alternately cauterizing and excavating the surfaces of the cavities, he came ultimately to the deepest layer of carious dentine, and cultures from this area yielded only three organisms, a streptococcus (**Streptococcus brevis**), **Staphylococcus albus**, and a Gram positive bacillus which he named **Bacillus necrodentalis**. Kantorowicz attacked the problem in the same manner and completely confirmed Goadby's findings. He found two types of streptococci, one at times pathogenic for mice. Both showed the morphological characters of **Streptococcus viridans**, i. e., growth in pairs and a tendency to become elongated. Two out of 14 strains were hemolytic, but the author does not believe that hemolysis is a satisfactory basis for classification. The streptococci were the most numerous. The staphylococci were always present in small numbers; they belonged to the non-pathologenic, non-liquefying white variety. The **Bacillus necrodentalis** apparently belongs to the lactic acid group of bacteria; it proved pathogenic for mice.

Similar, and even more striking, results were obtained by Niedergesäss, and published in a more recent article. In the deeper layers of carious dentine, streptococci alone were found, excepting in two cases where the **Staphylococcus aureus** grew. Of twenty-three strains of streptococci isolated, twenty he classified as **Streptococcus pyogenes**, since they formed long chains and hemolytic colonies on blood agar plates, while the individual cells were round; three strains he identified with the **Streptococcus acidi lactici** of Kruse, since they formed short chains, did not dissolve blood, and had elongated or oval cells. (It has been shown by various authors that the streptococci responsible for the normal souring of milk have the general properties of the group we designate as **Streptococcus viridans.**) Very few of his strains exhibited any pathogenicity for mice, and then only to a slight degree. The author also studied further the acid production of these streptococci in carbohydrate media, and in a comparative study showed that the streptococci produced as much acid as the other organisms in the outer layers of the carious dentine, and frequently more.

We must believe, then, that streptococci are the microbic agents that cause dental caries, whatever other factors may enter into the etiology. The fact that they are the sole organisms found constantly in the advancing border of the carious process indicates that they possess an **invasive power** not exhibited by the other bacteria found in cavities. It may be that the solution of the lime salts is accomplished by acids formed from other bacteria, altho mouth streptococci are powerful acid formers; it is quite certain that the digestion of the organic matrix is accomplished by the proteolytic activities of such saprophytes as **B. proteus, B. mesentericus,** and certain of the anaerobic bacilli. But these processes are secondary. The penetration and necrosis of the dental tissue, seen in the advancing border of the process, is very evidently due to the streptococci, and the streptococci alone.

Since a chronic alveolar abscess is usually consecutive to pulpitis produced by caries, it is reasonable to expect that here too, streptococci will be found the most important organism, and such, in fact, is the case. It is not easy to obtain cultures from deep in the jaw without contamination from the surface of the mouth, and such contamination is evident in most of the literature on this subject, which describes a great variety of organisms; most stress has been laid, however, on the pyogenic cocci, particularly staphylococci. Preceding our own studies on the bacteriology of chronic alveolar abscess, the only investigators who obtained similar results were Mayrhofer*, and Gilmer and Moody. The former author found streptococci in 47 of 50 cultures, in 30 cases in pure cultures, in others together with staphylococci and bacilli. He believes that the staphylococci are secondary invaders, and that the bacilli are without pathogenic action. Gilmer and Moody found the streptococci to be the most numerous organisms, but found other cocci and fusiform bacilli as well; they studied both acute and chronic abscesses and found hemolytic streptococci as well as the **Streptococcus viridans.**

Our own studies, published in previous papers, indicate that the **Streptococcus viridans** is the only organism found **constantly in chronic** alveolar abscesses, and in the great majority of cases is found in pure culture. Like the authors just quoted, we have at times found other organisms, particularly the white staphylococci and fusiform bacilli, but we believe that these are secondary invaders and practically devoid of pathogenic action. From acute abscesses we have recovered hemolytic streptococci on one or two occasions, but the majority of our cultures, from lesions where free pus occurred, have yielded staphylococci (also hemolytic at times) and the fact that these staphylococci have usually been found associated with the mouth streptococci, together with histological findings in the affected tissues, lead us to believe that many of the acute abscesses indicate a secondary invasion by staphylococci with a suppurative process implanted upon the primarily granulomatous lesion. Occasionally purulent infections are found where other organisms are apparently the etiological factor, such as the **Pneumococcus**, diphtheroid bacilli, **Bacillus pyocyaneus, Bacillus fecalis alkaligenes**, etc.; such infections are not, common, nor can they be considered characteristic dental lesions.

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*We have just recently become acquainted with Mayrhofer's work, and for that reason have not quoted it in our previous papers.

The literature on the bacteriology of pyorrhea is in hopeless confusion. All of the previous investigators, as far as we can determine, have dealt with the organisms in the free pus found in pockets, or even on the surface, and naturally they have obtained all of the organisms that occur in the mouth. Such studies are worthless as evidence for the etiological relationship of the organisms to the disease. We have followed a more rational technic. By thoroly cleansing the surface of gum and tooth, drying the area so cleansed and packing it off with dry sponges; by painting the area with iodine; by burning with the actual cautery the superficial tissues of the pocket; and then, having sterilized the field and removed the superficial layers of tissue with their surface bacteria, we have made cultures from the deep tissues and have usually obtained pure cultures of streptococci. That is, we have applied to the peridental membrane the principle involved in the technic which Kantorowicz and Niedergesäss used in studying the bacteriology of the dentine, and we have arrived at similar results and conclusions. The organisms found in the advancing border of the active disease process in pyorrhea alveolaris are streptococci; other organisms are found, staphylococci and occasional bacilli (especially fusiform bacilli) as in caries and in alveolar abscess, but they are not constant, and play a secondary role.

Whence come these streptococci, and how do they arrive at the tissues which they infect? In pyorrhea and caries the process begins so manifestly on the mouth surface that there is no question but that the organisms penetrate the tissues from the mouth. Pulpitis and alveolar abscess are usually secondary to caries, and may be considered in the majority of cases an extension of the same process. But the frequent occurrence of abscesses at the roots of artificially devitalized teeth, even where proper antiseptic measures have been used, and the finding of gangrenous pulps and abscesses in non-carious, apparently sound teeth, together with the occurrence of abscesses at the apices of entirely vital teeth has given rise to the view that many, if not all alveolar abscesses are produced by hematogenous infections. This theory, by no means new, was revived and exploited to a considerable extent recently in connection with the widespread interest in the focal infection theory. Under the influence of Rosenow's teachings concerning the mutation of streptococci and their elective localization, Ulrich, particularly, advanced the theory that "blind" abscesses result from the localization of streptococci that have invaded the blood stream, at a "locus minoris resistentiae" produced by the devitalization of the tooth; and Rosenow has published some remarkable experiments that seem to indicate that streptococci in diseased pulps actually develop an affinity for that tissue. While it is not within the scope of our paper to enter into a discussion of root canal treatment or the possible dangers of devitalization, it is necessary to the development of our thesis to show that dental infections are the result of direct invasion of the teeth or their investing tissues by streptococci from the mouth. We shall therefore digress a little to offer reasons why we believe the hematogenous theory is untenable. We cannot readily believe that the blood stream is frequently invaded by streptococci with no other symptoms or lesions than a dental abscess; yet probably the majority of persons who carry blind abscesses have no other evidences of a streptococcus infection. Not all devitalized teeth develop abscesses; Ulrich's figures, which are the highest we have seen, claim 70%. Granted that a perfect aseptic technic has been observed in the devitalization, and that the root canal has been thoroly disinfected, there yet remain other channels for reinfection than the blood stream, which we believe can be more rationally considered the true portals of entry for the streptococci. In the first place, Niedergesäss has shown that in many teeth there occur microscopic clefts in the dentine, thru which organisms from the mouth can pass directly into the pulp chamber, without caries; these may explain cases of pulpitis without caries. Secondly, Mayrhofer has shown that in pulpitis there occurs an invasion of the dentinal tubules, especially at the apex, and that within two weeks after disinfection and filling of the root canal, cultures from the pulp chamber will again yield streptococci; this type of reinfection, we believe, deserves serious consideration. Finally, we believe that the lymphatic system of the alveolar process is an important factor. We have repeatedly found that cultures from periapical tissues of healthy teeth two or even three teeth removed from an abscess yielded streptococci, and this can only be explained as a spreading of the infection thru the regional lymphatics. Such an invasion by way of the lymphatics must also take place frequently from the tissues about the gingival crevice, passing directly down the peridental membrane; we have found areas of gingivitis and alveolar periostitis in microscopic sections which were not at all visible grossly, which could well serve as foci of lymphatic distribution to deeper parts of the peridental membrane. Such lymphatic invasion will explain abscesses on vital teeth.

It is unfortunate that those investigators who have preceded us in the study of streptococci in dental lesions have failed to observe accurately those qualities by which streptococci are now classified, namely, hemolysis and carbohydrate fermentations. Siebert and Mayrhofer offer nothing in this line. Goadby identified the streptococci of caries with the normal mouth streptococcus (which he calls **Streptococcus brevis**) by morphological and general cultural characters. Kantorowicz made no classification, but his morphological descriptions together with the fact that twelve out of fourteen strains were non-hemolytic indicate that he, too, was dealing with the same kinds of streptococci as are found in the normal mouth. Niedergesäss, alone, found a predominance of long chained hemolytic streptococci. We have studied both hemolysis and carbohydrate fermentations with a long series of strains from pyorrhea and alveolar abscess, and have found that, with one or two exceptions, all the streptococci from these lesions are identical with the normal mouth streptococcus, the **Streptococcus salivarius** of Andrewes and Horder. This, we believe, is the strongest evidence that the streptococci in dental lesions come directly from the mouth, and not from a distance by way of the blood stream.

We have thus shown that streptococci alone are found constantly in the deepest layers of diseased tissue in caries, pulpitis, chronic alveolar abscess, and pyorrhea, the common infectious diseases of the teeth and their investing tissues, or, otherwise stated, the streptococci show locally in the mouth greater invasive power (pathogenicity) than any of the other mouth bacteria that have been associated with the diseases mentioned.

It is not necessary for us to quote extensively from the literature on metastatic infections produced by mouth streptococci. The identification of the **Streptococcus salivarius** with the **Diplococcus rheumaticus** of Poynton and Paine and with the **Streptococcus viridans** of Schottmüller has definitely associated this organism with the various lesions that go to make up acute and chronic rheumatism, particularly **endocarditis.*** The frequency with which these lesions occur in man is evidence that the mouth streptococci exhibit an ability to invade the blood stream equal to the **Streptococcus pyogenes,** and not shown by any of the other mouth bacteria. Unlike the **Streptococcus pyogenes,** the members of the viridans group are unable to maintain a continued existence in the blood, and seldom produce septicemia, but tend rapidly to localize. The focal lesions in the tissues, produced by these streptococci, are moreover, seldom purulent in character; instead of producing acute inflammations with abscess formation, as does the **Streptococcus pyogenes,** the mouth streptococci produce in focal lesions a subacute or proliferative type of inflammation with lymphocytes and plasma cells, or endothelial cells and fibroblasts as the principal cells of the exudate, rather than pus cells. That is, while the mouth streptococci show an invasive power equal to the hemolytic forms, they do not form so potent a toxine, and are therefore less virulent. We wish to emphasize the inability of the Streptococcus viridans to produce pus in the human body, and to offer this fact as further evidence to prove that this organism is primarily responsible for the infections of the peridental membrane, since it has been shown by various pathologists, particularly in Germany, that neither pyorrhea nor chronic alveolar abscess are primarily suppurative, but are granulomatous in character, and that in both of these diseases plasma cells and lymphocytes are the principle inflammatory cells.

We need not, either, devote much space to the question of animal inoculation experiments. We have reviewed the literature and presented the results of our own experiments in previous papers. Such experiments have been performed so often by so many different investigators that there can remain no doubt that mouth streptococci, when injected into rabbits, show a degree of pathogenicity not equalled by any of the other common mouth bacteria. In our first reports we showed that mouth streptococci are capable of producing in rabbits lesions identical with those produced by strains of **Streptococcus viridans** obtained from the lesions of rheumatism and endocarditis. In a later report we determined more accurately the degree of pathogenicity which **Streptococcus viridans** (particularly the mouth strains) exhibits for rabbits, and compared their virulence with that of the hemolytic **Streptococcus pyogenes.**

Fortunately, the period of exploitation of the focal infection theory is about over; the dental profession has awakened to new responsibilities and new opportunities, while the medical profession is beginning to rediscover the fact that there are other causes of disease and other portals of infection than the dental abscess. There are still rife, however, many speculations and unproven theories as to the possible dangers of peridental infections and their ability to produce a wide variety of systemic diseases. We feel, therefore, that we should state here our opinion upon this subject. We have noted several cases where mouth streptococci from dental abscesses have invaded the lymph glands of the neck and have produced a chronic non-suppurative lymphadenitis, with cellulitis in one case, and two cases where strains of high virulence invaded the blood stream and produced death from septicemia; but aside from these non-specific types of infection which may at times be produced by any organism, we do not believe that mouth streptococci have been proven to cause any other systemic disease than the various lesions that go to make up the disease known as rheumatism, and we believe the dental lesions are more particularly the source of the chronic progressive forms of this disease. We have been misinterpreted by various people as claiming that streptococci from dental abscesses in patients suffering from rheumatism show a specific tendency to produce rheumatic lesions in rabbits not exhibited by mouth streptococci from other individuals. We have, however, not made any such statement, and, as a matter of fact, have found all mouth streptococci, whether from diseased individual or normal, showing a tendency to produce rheumatic lesions in rabbits. We have stated that streptococci from dental abscesses are more pathogenic than those from normal saliva, which is of course to be expected.

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*The studies of Poynton and Paine, and others, have established that most forms of heart disease are a part of the rheumatic process, and they are so considered by us.

We are unable to accept, in any degree, Rosenow's elective localization theory. We have seen no evidence in our own experiments to confirm it, altho it is but fair to state that we have not used his technic, feeling that, particularly in the inoculation of rabbits with enormous doses of **mixed cultures**, it is not good bacteriological practice. Moody, however, has studied this question very thoroly, working with streptococci from dental abscesses and using essentially Rosenow's technic; cultures were obtained from dental abscesses in 15 patients with articular rheumatism, 15 patients with other forms of systemic disease, and 19 patients healthy save for their mouth infections, and in all 178 rabbits were inoculated. The percentages of lesions in the various tissues of the rabbits are compared and tabulated. He concludes that "the lesions produced by organisms from persons without systemic disease are identical in character with those produced by organisms from patients with systemic infections. The fact that the former do not produce lesions quite as frequently as the latter indicates a difference between the organisms only in degree of virulence," and further that "the localization of **Streptococcus viridans** cannot be determined wholly by some property inherent in the organism itself."

We wish to call attention to the fact that members of the **Streptococcus viridans** group are normally present thruout the gastro-intestinal tract as well as in the mouth. Failure to recognize this fact has led to two sorts of errors in the focal infection theory. Firstly, the fact has been overlooked that **Streptococcus viridans** may invade the blood stream from the intestines as well as from the mouth, and the presence of **Streptococcus fecalis** (mannite fermenting) in a goodly number of cases of endocarditis indicates that infections from this source do frequently occur; that is, focal lesions of the intestines must be considered as well as lesions about the teeth and tonsils in trying to find the source of infection in systemic diseases. Secondly, the finding of **Streptococcus viridans** in ulcerative lesions of the gastro intestinal tract (gastric ulcer, cholecystitis, appendicitis) has given rise to the theory that such lesions are produced by a localization of streptococci from focal infections in the mouth which have invaded the blood stream, whereas such streptococci are normally present on the mucous surfaces in question; and the failure of the investigators of these diseases to differentiate the streptococci found, both in clinical observation and in animal experiment, by carbohydrate fermentation tests, leaves one in doubt as to whether these streptococci had actually come from the blood stream or had been originally present on the surfaces of the ulcerated mucous membrane.

How dangerous are dental abscesses to the general health? These lesions become definitely encapsulated by scar tissue, and frequently undergo spontaneous healing, while the streptococci that cause them are of very low virulence. Nevertheless, the fact that these abscesses remain unhealed or slowly progressive over a long time indicates that the balance between virulence of organism and resistance of host is very delicately maintained, and may be disturbed in favor of the streptococci by very slight influences. While the organisms are of low virulence, this is due to the low grade character of their toxines and not to any lack of ability to invade tissues. Moreover, they show a great tenacity, being able to maintain an existence in the body for some time after invasion. We believe that the streptococci produce no immunity, but tend rather to break down the normal resistance. This is indicated both by the generally admitted failure of vaccine therapy, and by the fact that infections are usually followed by repeated recurrences of increasing severity.

We stated at the beginning of this paper that since dental infections are produced by organisms normally present in the mouth, there must be other factors in the etiology of these diseases than the mere presence of bacteria, factors which break down the resistance of the tissues and allow the organisms to invade. We may now apply this statement more specifically to the streptococci. Some of these factors are well defined from clinical observation. They may be general in character, like mercurial (and possibly phosphorous) poisoning, and scurvy, diabetes and pernicious anemia; or local, as mechanical injury, accumulations of tartar, etc. We cannot reproduce dental infection by merely introducing streptococci into the mouths of animals, or even by inoculating them into the peridental tissues; but we can experimentally reproduce some of the conditions that lead to dental infections. Jackson and Moody have shown that, by altering the diet of guinea pigs, experimental scurvy may be produced, and that, in the mouth lesions as well as the internal lesions of the animals so diseased, the **Streptococcus viridans** is found. We have found that by feeding cats with calomel we can produce an experimental mercurial stomatitis, with loosening of the teeth and the formation of abscesses in the jaw, and from the depths of the lesions so produced, as well as from the cervical lymph nodes, we have isolated streptococci. (The mouth flora of cats is very similar to that of man.) By cutting off the crowns of teeth in cats and exposing the pulp to the saliva, we have produced alveolar abscesses which contained streptococci in pure culture; and by placing wire ligatures about the necks of the teeth and so irritating the gingival tissues, we have produced a typical pyorrhea. We believe that a great deal concerning the etiology and pathogenesis of dental infections, as well as their role in the production of systemic disease may be learned from a large series of such experiments.

## SUMMARY AND CONCLUSIONS.

In the preceding pages we have reviewed the more important literature on the bacteriology of the mouth, with particular reference to dental diseases, and gathered together certain data to confirm the following conclusions:

1. Mouth streptococci are the only true parasitic organisms of the normal, characteristic mouth flora, since they alone are capable of maintaining existence on the clean healthy mucous membranes before the eruption of teeth brings conditions of uncleanliness and decay. They are the most numerous of the mouth bacteria.

2. Mouth streptococci are only relatively decreased in the mouth in conditions of uncleanliness and decay; they may be absolutely increased. This relative decrease cannot be accepted as evidence that streptococci do not cause the dental diseases that follow uncleanliness in the mouth.

3. Mouth streptococci are the microbic causes of dental caries, pulpitis, alveolar abscess, and pyorrhea alveolaris, because:

a. They are the only organisms found commonly in these lesions, which are of proven pathogenicity, as exhibited by local invasive power, ability to invade the blood and lymph streams and so produce metastatic infections, and by the results of animal inoculation experiments.

b. They are the only organisms found constantly in the deep layers or advancing borders of infected tissue in the diseases named; they are frequently found in these areas in pure cultures.

c. In metastatic infections and in experimentally inoculated animals they produce subacute and granulomatous lesions similar to the inflammatory reaction found in pyorrhea and in chronic alveolar abscess.

4. The streptococci found in dental diseases are the true mouth streptococci (**Streptococcus salivarius**) and invade the dental and peridental tissues from the mouth.

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# Liberation of Antibodies on Injection of Foreign Proteins

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# LIBERATION OF ANTIBODIES ON INJECTION OF FOREIGN PROTEINS *

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Many reports have been published concerning the treatment of acute and chronic infections by the intravenous injection of foreign proteins. This treatment has been applied to typhoid fever, arthritis, and certain skin diseases. It apparently developed as an outgrowth of the injection of vaccines. Vaccine therapy, once the great hope of modern therapeutics, failed to produce the promised results, and, with the exception of prophylactic vaccination against typhoid, variola, rabies, and perhaps in acne and allied pyogenic skin diseases, was being abandoned more and more. Now there is an apparant revival of allied therapeutic methods in the form of foreign protein injections.

The beginning of foreign protein therapy dates back to the work of Fraenkel[1] in 1893, who treated 57 cases of typhoid fever with subcutaneous injections of typhoid vaccine. He reported that most of the cases were favorably modified, and that a few terminated by rapid lysis. This use of typhoid vaccine during the active, acute stage of the disease was a new departure, which demanded further investigation.

Rumpf,[2] using a vaccine of Bacillus pyocyaneus, repeated the work in another group of cases, and observed equally favorable results.

Ichikawa,[3] using intravenous injections of sensitized vaccine, and Kraus and Mazza,[4] using polyvalent vaccine, were able to produce favorable modifications in 60% of their cases. They also observed equally good results in paratyphoid and found that colon vaccine could be substituted for the typhoid vaccine.

More recent work on typhoid has been reported by Lüdke[5] with albumoses; Saxl,[6] using a solution of caffein and camphor; Kibler and McBride,[7] using typhoid vaccine, etc.

Foreign protein has been used also in the treatment of arthritis. Notable is the report of Miller and Lusk.[8] Miller later summarizes his results and

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[1] Deutsch. med. Wchnschr., 1893, 19, p. 985.

[2] Ibid., 1893, 19, p. 987.

[3] Ztschr. f. Immunitätsforsch. u. exper. Therap., 1914, O., 23 p. 32.

[4] Deutsch. med. Wchnschr., 1914, 40, p. 1556.

[5] München. med. Wchnschr., 1915, 62, p. 321.

[6] Wiener med. Wchnschr., 1916, 66, p. 115.

[7] Jour. Infect. Dis., 1917, 21, p. 13.

[8] Jour. Am. Med. Assn., 1916, 66, p. 1756 and 1916, 67, p. 2010.

gives an extensive bibliography of work done by other clinicians.[9] The results as reported appear promising, especially in acute arthritis.

Other favorable results have been reported in skin diseases, puerperal sepsis, conjunctivitis, iritis, trachoma, typhus, tuberculosis, etc. Among the variety of proteins employed are: colon vaccine, chicken serum, whole blood, proteose, albumose, horse serum, and autolyzed vaccine.

Closely related to the response to foreign protein is the old and familiar observation that a specific disease may sometimes improve with the advent of some intercurrent infection. For example, the symptoms of acute gonorrhea have been observed to subside with the incidence of typhoid fever. Attention has also been called to the increased resistance of the body to various common infections, following any vaccine injection.

It is evident that foreign protein therapy is empirical and non-specific. Almost any foreign protein seems to be effective, and may be employed more or less successfully, under suitable conditions, in a variety of diseases. The question arises as to what factor is responsible for the apparent benefits observed. If this manner of treatment is to be placed on a scientific basis this factor must be determined. Clinical investigations have thrown no light on this question. The type of reaction is apparently the same in all cases.

As described by Kibler and McBride[7] the reaction of a typhoid patient to the intravenous injection of typhoid vaccine is as follows:

1. *Temperature.*—Within a few minutes to 1 hour after the injection a chill occurs, lasting 20-30 minutes. This is followed by a rapid rise of temperature which later falls abruptly, and then tends to remain remittent in favorable cases.

2. *Leukocytes.*—Usually there is an early leukopenia within 4 hours, due mainly to a disappearance of polymorphonuclears. Within about 12 hours this is followed by a moderate polymorphonuclear leukocytosis.

3. *Blood pressure* is usually decreased by 10-15 mm. after the chill.

4. *Coagulation Time.*—Only slight and insignificant changes have been observed.

5. *Antibodies.*—No essential difference from the specific response to the injection of typhoid antigen in a normal individual was observed in these typhoid patients. It is to be noted, however, that conditions here differ somewhat from those in which an heterologous protein is injected.

The reaction in arthritis cases agrees essentially in the first four particulars with the reaction in typhoid fever. The antibody response will be considered later. Most clinicians seem agreed in regarding the severe chill as the most important factor in this reaction. It is evident that a chill alone cannot be responsible for the marked relief from pain and for the remission in the course of an acute arthritis. It is possible, however, that the resulting hyperpyrexia may have an effect in favoring immune reactions and antibody production.

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[9] Miller: Jour. Am. Med. Assn., 1917, 69, p. 765.

Rolly and Meltzer,[10] in a study on the effect of high temperatures, injected a series of rabbits with small doses of typhoid bacilli or cholera vibrios. Some of these rabbits were kept in an oven so that their body temperature remained between 40 and 42 C. The controls were kept at ordinary room temperature. They observed a higher average production of agglutinins and lysins in the high temperature rabbits than in the controls. They also showed that phagocytosis by human leukocytes in vitro occurs at its optimum between 37 and 40 C.

More recently, Winslow, Miller and Noble[11] investigated the effect of moderately high temperatures on the production of lysins against sheep blood corpuscles. They kept their rabbits in an oven at a moderately high temperature, 29 to 32 C. Injections of sheep cells were given and the hemolytic titer estimated each week. They found that the rate of lysin production in the heated rabbits was slower than that in the controls.

These experiments may indicate that a moderately high temperature lowers the general vital resistance to a beginning infection. Once an infection is established, however, according to Rolly and Meltzer's results, hyperpyrexia may bring about a greater production of antibodies.

If a specific leukocytosis could be observed, one might think that this would be a factor in overcoming the infection, but the degree of leukocytosis seems only to parallel the severity in each case.[12] Gay and Claypole[13] believed that a specific hyperleukocytosis occurred in typhoid immune rabbits, following injections of typhoid bacilli, but McWilliams[14] later showed that both normal and immune rabbits respond to about the same degree. The leukocytosis therefore cannot be regarded as specific.

The slight variations in blood pressure and coagulation time are evidently insignificant.

We pass now to a consideration of the antibody response. Very little work on this aspect of the question has been done, yet it seems as if the explanation of a therapeutic response, in the case of any infection, to the injection of a substance not specifically bactericidal to the infecting organism, should logically be sought in the liberation of specific antibodies against that organism. Ordinarily when the body overcomes a streptococcic infection, for example, we believe this is accomplished by the production of antibodies against the streptococcus. Why, then, should we not suspect that the therapeutic effect of foreign protein injections in infections, obviously not due to the foreign protein per se, has as its basis the liberation of specific antibodies against the causative organism? In the case of arthritis we believe that the symptoms persist because the antibodies which should normally be produced are for some reason not liberated.

In the light of Ehrlich's receptor theory, we recognize three distinct phases in the production of antibodies: (1) the sensitization of the tissue cells; (2) an overproduction of sessile receptors; (3) the liberation of these receptors from the cells. A perfect antigen is one which causes all of these phases to be completed. It seems a plausible hypothesis that in those cases of arthritis, etc., which show a rapid therapeutic response to injections of foreign protein, the etiologic organism is acting only as an imperfect antigen. It sensitizes the cells and causes sessile receptor production, but does not give the necessary stimulus to cause these receptors to be thrown off. The foreign protein then supplies this stimulus, the system is flooded with specific antibodies, and a remission of the infection results. Although a variety of proteins may serve to complete this second phase, the reaction is still absolutely specific, since there must be a specific stimulation of cells, and a final liberation of specific antibodies.

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[10] Deutsch. Arch. f. klin. Med., 1908, 94, p. 335.

[11] Proc. Soc. Exper. Biol. and Med., 1916, 13, p. 93.

[12] Cross: Journal-Lancet, 1917, 37, p. 764.

[13] Arch. Int. Med., 1914, 14, p. 662.

[14] Jour. Immunol., 1916, I, p. 159.

In this connection I would recall the experiments of Bruck.[15] According to Ehrlich's theory a molecule of tetanus toxin is composed of a haptophore group, which unites with the cell receptor, and a toxophore group, which is responsible for the poisoning of the cell. This toxophore group can be destroyed by various methods such as heat, light, and long preservation. The toxin has then become a toxoid. The haptophore group can still unite with a sessile cell receptor, or with a specific antibody, but because of the absence of the toxophore group the molecule has become innocuous. Bruck injected rabbits with small repeated doses of such a toxoid. The rabbits were found to produce very little, or no free antitoxin, the amount being dependent on the amount of effective toxophore groups which still remained in the toxoid. He further showed that rabbits injected with tetanus toxoid, during the first few succeeding hours possessed a greater tolerance for tetanus toxin, but that later they showed an actual hypersensitiveness, being killed by only a fractional portion of the ordinary lethal dose of toxin.

These results Bruck interpreted as follows: The haptophore group of the toxoid at first is anchored to the specific cell receptor. The toxin molecule introduced soon afterward, therefore, cannot reach the cell, since all the receptors are blocked. Later, however, an overproduction of sessile receptors occurs, and a hypersensitiveness to toxin results, since the avenues of approach to the cell are increased many times. The toxoid has acted as an inferior antigen. It has brought about the first and second steps of antibody production, namely, (1) sensitization of cell receptors, and (2) the overproduction of similar sessile receptors, but it has lacked the necessary stimulus to cause the discharge of these receptors. Had the rabbits been immunized with small doses of unchanged toxin this third stage would have been completed. Bruck therefore sees in the toxophore group the necessary stimulus for the setting free of the receptors. Might not a foreign protein, in an analogous way, supply such a stimulus? It was with this hypothesis in view that I undertook an investigation of the effect of foreign protein on antibody production. The experiments have been conducted entirely with rabbits, because all the factors can thus be much more accurately controlled than in a purely clinical investigation.

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[15] Ztschr. f. Hyg. u. Infections-Krankh., 1904, 46, p. 176; 48, p. 113.

Several other investigations have been reported which touch on the immune reactions in foreign protein therapy.

Jobling and Petersen[16] report a "mobilization of ferments," notably protease and lipase, following foreign protein injections. Later[17] they point out an "increase in antiferment," dependent on "highly dispersed, unsaturated lipoids," and conclude that "the main therapeutic factor is probably an increased dispersion of colloids."

Culver[18] investigated the opsonin and lysin content in the serum of patients with gonorrheal arthritis, after intravenous injections of gonococcic vaccine and proteose. He reports a rise in titer of these antibodies.

Bull[19] reports a rise in the antibodies of typhoid immune rabbits after the injection of typhoid bacilli; but his results could not be confirmed by Teague and McWilliams,[20] who suggest a new theory to explain the therapeutic effect on cases of typhoid, of intravenous injections of typhoid vaccine. On the supposition that blood serum is more bactericidal than lymph, they base the theory that the vaccine causes a greater flow of bactericidal serum into the lymphoid organs, and thus overcomes the local infection. It does not appear that this theory has been substantiated.

On the basis of the hypothesis that the foreign protein reaction serves as a stimulus to set free specific antibodies, I first investigated opsonins for the streptococus. This organism is notably a poor antigen, in that it is difficult to obtain a high antibody titer against it. An animal sensitized with this organism should therefore be a favorable subject for the liberation of antibodies by foreign protein.

*Series* 1.—Two rabbits were injected intravenously at 3-day intervals with 1 c c of a fresh suspension of killed Streptococcus viridans. The organism was grown on blood agar slants, and a 24-hour growth washed off with 10 c c salt solution. This emulsion was heated at 60 C. for 1 hour to kill the bacteria, and 1 c c of this vaccine used as a dose. A fresh vaccine was made each time. The fourth injection consisted of ½ c c of living streptococci. Five days later the opsonic index was determined, and 2 c c of human ascitic fluid injected intravenously.

The method of determining the opsonic index was that of Wright, using the experimenter's own leukocytes and serum as a standard.

Both rabbits were found to have a very low opsonic index 5 days after the fourth injection of streptococci; 2 c c of human ascitic fluid were then injected, and a marked rapid rise of opsonins occurred, the total increase being 700% in 48 hours. Apparently the foreign protein acted as an effective stimulus to liberate sessile receptors.

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[16] Jour. Am. Med. Assn., 1915, 65, p. 515.

[17] Ibid., 1916, 66, p. 1753.

[18] Jour. Lab. and Clin. Med., 1917, 3, p. 11.

[19] Jour. Exper. Med., 1916, 23, p. 419.

[20] Jour. Immunol., 1917, 2, p. 185.

After 18 days the opsonic content was again found to be at a low level. Two further injections of streptococci were then given. This time an interesting variation in response occurred. The opsonic index of Rabbit 17 rose, while that of Rabbit 311 continued to fall. In the former the streptococci evidently acted as a perfect antigen, causing all three stages of antibody production, while in the latter the final liberation of antibodies did not take place. This supposition was confirmed by the effect of an injection of ascitic fluid. In Rabbit 17 there resulted no further increase in opsonins. The stimulus of the antigen had been sufficient to liberate all antibodies. In Rabbit 311 a rapid liberation of opsonins followed the foreign protein injection. In this case we picture the tissue cells as loaded with an overproduction of

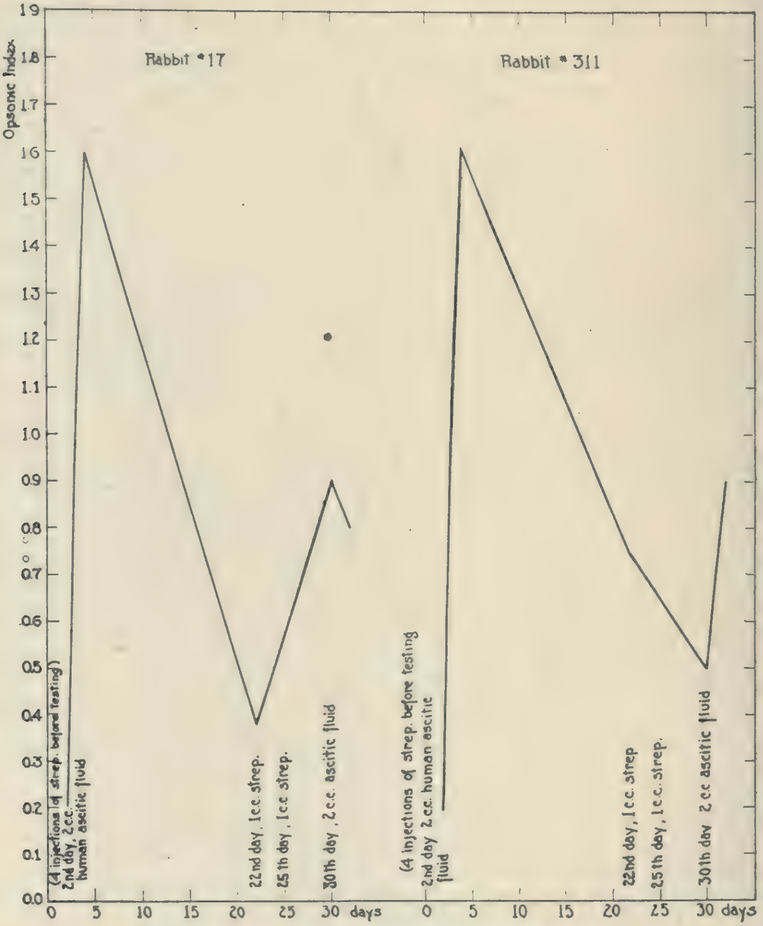

Chart 1 (Series 1).—Opsonins for streptococcus.

specific sessile receptors. The foreign protein reaction then acted as an effective stimulus to cause the cells to throw off these receptors.

*Series 2.*—In this series 2 rabbits were injected with fresh suspensions of meningococci, and human serum was used as foreign protein. Rabbit 395 received 2 injections of serum with a resulting rise after each injection. Apparently the cells were not completely desensitized by the first serum injection. Rabbit 268 received no second injection and showed a gradual decrease in opsonins.

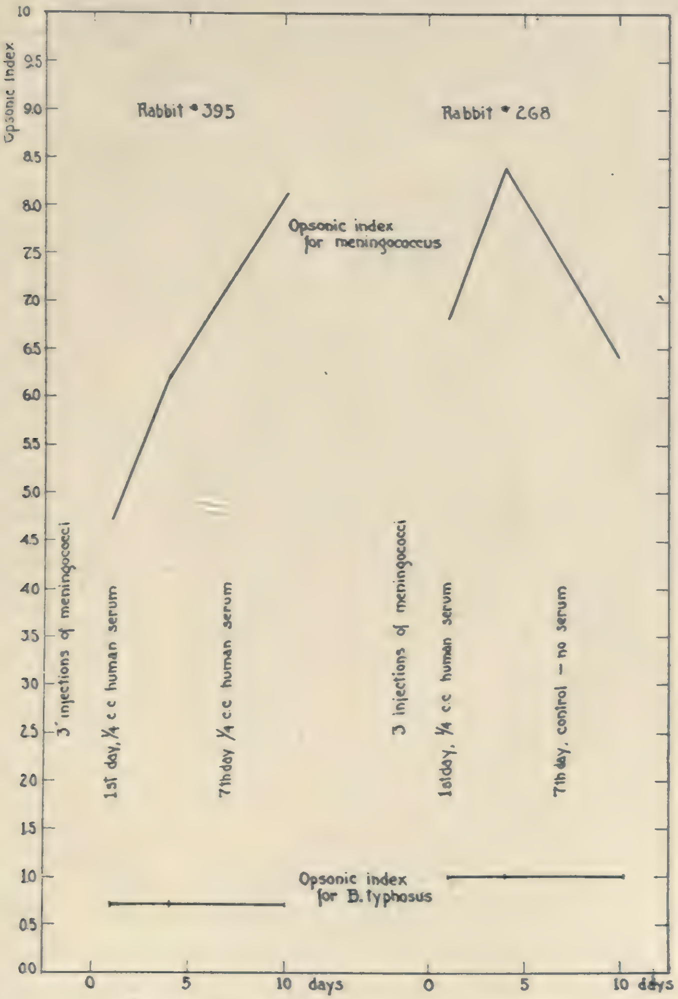

Chart 2 (Series 2).—Opsonins for meningococcus.

In order to demonstrate whether these antibodies were specific for meningococci, and also as a control of the technic, the serums were also tested each time for opsonins against B. typhosus. The typhoid opsonic index remained low and unchanged throughout.

*Series 3.*—Two rabbits were given 4 intravenous injections of S. viridans at 3-day intervals, as in Series 1. Six days after the fourth injection the opsonic index was determined. Rabbit 31 then received 1 c c of human serum intravenously, while Rabbit 32 was used as a control and received no serum. The former showed a rise of 38% on the third day following, while the opsonic index of the latter remained stationary. The control test for B. typhosus remained unchanged in both cases.

During the next 40 days 4 injections of a dextrose broth culture of streptococcus were given. Six days after the last injection the opsonic index was again determined. It was found lowered in both rabbits. Rabbit 32 then received 1 c c of human serum. There followed a rise of nearly 300%. Rabbit 31 showed a spontaneous rise, but to a much less degree. A check opsonic determination for meningococci showed no variation.

It was now thought that if an increase in opsonins occurred, following foreign protein injection, it should also be possible to demonstrate a simultaneous rise in the agglutinin titer for streptococci.

The agglutinin titer was determined by the microscopic method as follows: The rabbits were bled from the ear veins, the blood allowed to clot, and the serum separated by means of rapid centrifuging. Varying dilutions of the serum were then made in 0.9% salt solution. Hanging drop preparations were made by placing on a coverslip, with a small platinum loop, 1 drop each of diluted serum and of a 15-hour dextrose-broth culture of streptococcus. The coverslips were then inverted on vaselined hollow ground slides, and left at room temperature. Readings were then taken under the microscope at the end of 3 hours. Positive agglutination could be observed in the lower dilution, consisting of definite clumping of the streptococcal chains. The highest degree of dilution in which a positive test occurred was recorded as the agglutinin titer.

The first tests were made simultaneously with the last opsonic determinations. In response to the first serum injection Rabbit 32 showed a rise in agglutinin titer of 250%. Rabbit 31, which served as a control at this time, also showed a slight rise (50%) and continued to rise gradually. The agglutinin titer of Rabbit 32 began to fall after the third day. All the sessile receptors apparently had been freed by the foreign protein injection, while in Rabbit 31, without the stimulus of foreign protein, they were liberated to a less degree and more slowly.

In order to resensitize the tissue cells 2 c c of broth culture were now injected. Seven days later the agglutinin titer had fallen very low in both rabbits. This time Rabbit 31 received 1 c c of human serum, while Rabbit 32 served as control. A rapid rise of agglutinin titer occurred in the former which then remained stationary. In the control rabbit a more gradual rise occurred. Thus the specific response to the foreign protein injection was again observed.

*Series 4.*—It now became necessary to investigate whether or not foreign protein injections would serve to bring about a mobilization of antibodies against an organism which was known to be a good antigen, that is, one which when injected into an animal, would readily cause the production of a high titer of free antibodies. The typhoid bacillus is known to be such a perfect antigen, when injected into rabbits. Indeed, it is not unusual to obtain an agglutinin titer against typhoid as high as 1:500,000 dilution.

Two rabbits were injected repeatedly with emulsions of typhoid bacilli, until they showed an agglutinin titer of 1:50, on testing by the macroscopic method; 1 c c of guinea-pig serum was then injected and the agglutinin titer again determined in 48 and 72 hours. No rise in titer was apparent.

Further injections of typhoid bacilli were then given until the agglutinin titer reached 1:100. Then ½ c c of human serum was injected. Again no increase in agglutinins could be observed during the next 72 hours. A second injection of human serum did not change the titer.

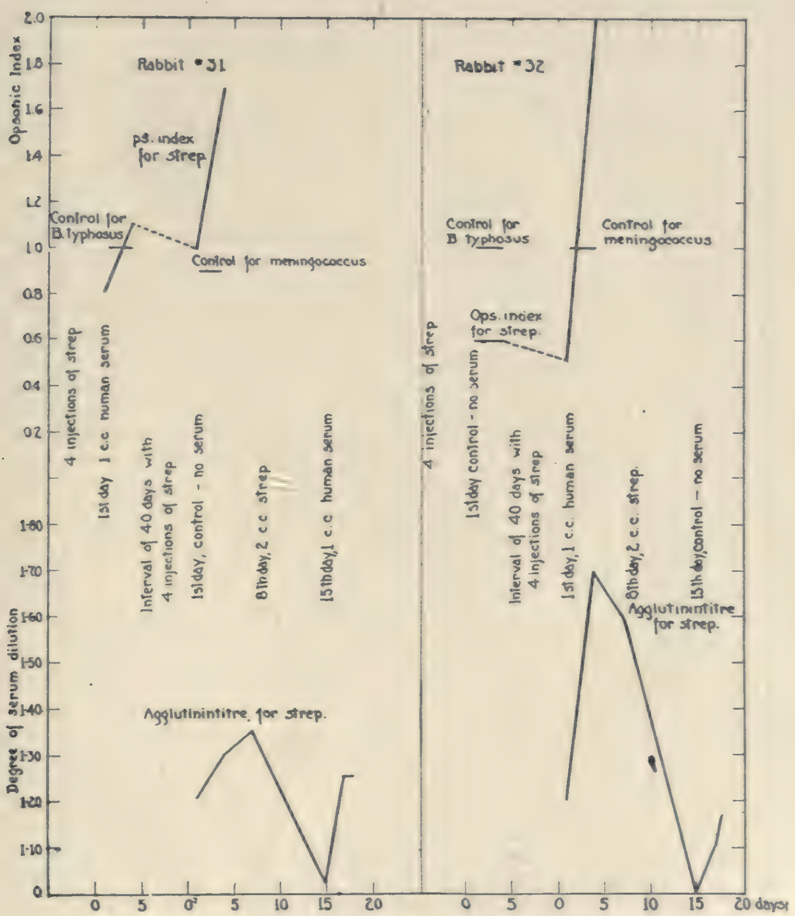

Chart 3 (Series 3).—Opsonins and agglutinins for streptococcus.

In order to check these negative results 2 more rabbits were immunized with typhoid bacilli. After 2 injections of bacilli the agglutinin titer was found to be zero, by the microscopic hanging drop method, as described under Series 3; ½ c c of human serum was then injected, and the antibody titer again determined on the third day following. Again no agglutinins were found. Evidently the rabbits had not yet been sufficiently sensitized against the bacilli.

Immunization with typhoid bacilli was then continued until the microscopic method revealed an agglutinin titer of 1:3,000. Two c c of human ascitic fluid were then injected intravenously. No rise in agglutinin content resulted.

The serum of these rabbits was also tested for complement fixing bodies, with the idea in mind that the foreign protein reaction might possibly bring about a liberation of such antibodies, although it apparently had no effect on the agglutinin content.

As an antigen in the complement fixation test an old suspension of typhoid bacilli in salt solution, to which 0.3% tricresol had been added, was used. The test was carried out with the usual technic, using this typhoid antigen, washed sheep blood cells, fresh guinea-pig complement, and rabbit amboceptor. The serum of the typhoid immune rabbits was inactivated by heating at 56 C. for ½ hour, then diluted, and the fixing power of a given quantity tested. It was found that the ability of the inactivated serum to fix complement was not changed in the slightest degree by foreign protein injections.

We must conclude that foreign protein injections have no effect on the antibody production in typhoid immune rabbits. The typhoid bacillus is apparently a perfect antigen in rabbits, and no foreign protein is needed to stimulate the liberation of antibodies against it.

How, then, can we reconcile this with the apparent therapeutic benefits observed in some human patients? May it not be possible that some patients do not react completely to the antigenic stimulation of the typhoid bacillus? In such cases the foreign protein might well serve as a stimulus causing the sensitized cells to throw off their sessile receptors. This sudden flooding of the system with antibodies might then be responsible for the remission in the disease. This hypothesis seems to be supported by the succeeding series.

*Series 5.*—Thus far I have recorded observations on opsonins and agglutinins. It seemed desirable also to investigate the response of a third type of antibodies, lysins, to foreign protein injections. Since it is difficult to make accurate determinations of the lysin content of a serum for a specific bacterial cell, we decided to use as our antigen washed sheep corpuscles. A hemolytic titer can be very accurately obtained, while a bacteriolytic titer, obtained by the method of extinction and plating, is modified by too many possible errors of technic.

It is well known that rabbits will readily produce lysins against sheep corpuscles. Common use is made of this fact in producing amboceptor for the hemolytic system of the Wassermann reaction. We may say, therefore, that sheep cells act as a good antigen when injected into rabbits. This would allow us to predict, on the basis of the preceding series, that foreign protein injection into rabbits sensitized against sheep cells would have little effect on the liberation of specific lysins. It has, however, been frequently observed in this laboratory that a great variation exists in the readiness with which different rabbits will produce amboceptor when injected with sheep cells. Rubinstein[21] recently studied the hemolytic titer of rabbits after 3 injections of sheep red cells. The titer was determined before each injection, and 7 days after the third. He found that of 11 rabbits only 8 showed a definite marked rise in lysins after each injection. In one of the remaining 3 the titer remained the same, and in the other 2 there was even a decrease in lysin content after successive injections. In such rabbits as the latter 3 the sheep cells evidently acted only as an inferior antigen, and in such cases we would expect, on the basis of our previous work, to be able to liberate antibodies by means of foreign protein injections.

[21] Campt. rend. Soc. de biol., 1917, 80, p. 908.

The hemolytic system in these experiments consisted of 0.10 c c fresh guinea-pig complement (diluted 1:10), 0.5 c c of a 2% suspension of fresh washed sheep corpuscles, 1.5 c c of 0.9% salt solution, and graduated amounts of inactivated rabbit serum. The least amount of serum that would cause complete hemolysis after incubation at 37 C. for 1 hour was recorded as the lysin titer.

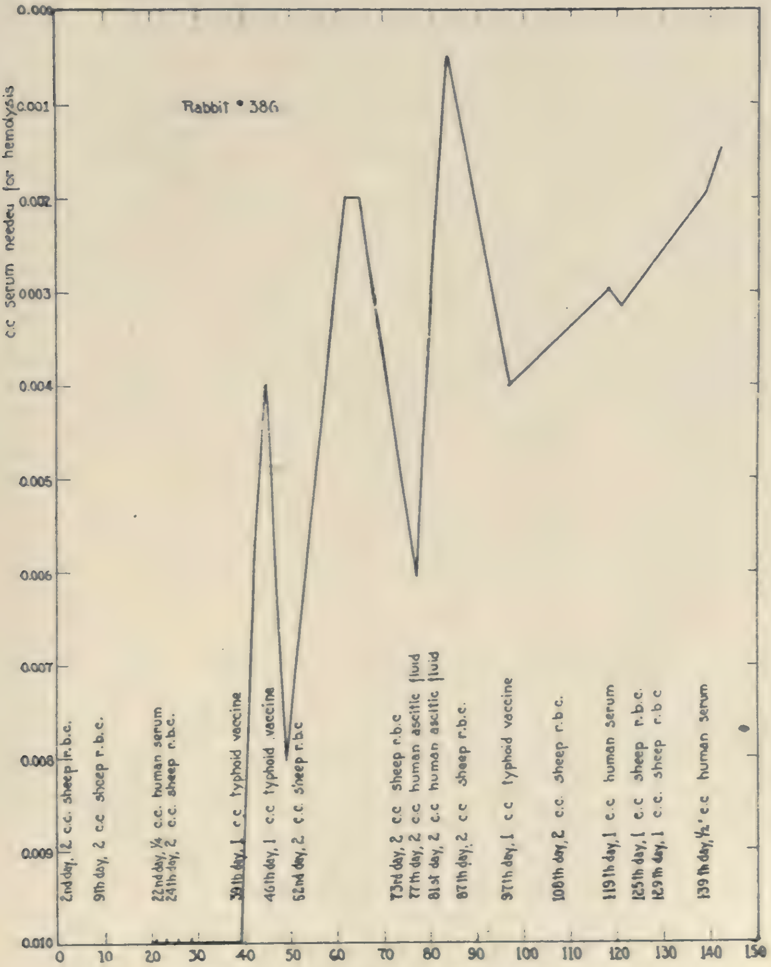

Chart 4 (Series 5).—Lysins for sheep cells.

A total of 20 rabbits was used in this series. The rabbits were injected intravenously with 2 initial doses of sheep corpuscles in salt solution. As a rule the lysin content was then measured, 10-12 days after the last injection, it having been determined that the lysin titer normally reaches its height by the end of this period. After foreign protein injections, either human serum or typhoid vaccine, the lysin content was again determined, usually in 72 hours, in order to give ample time for any resulting change in titer to take place.

In Rabbit 386 sheep cells evidently acted as an inferior antigen, the lysin content 11 days after the second injection being very low and remaining so; 0.25 c c of human serum injected on the 17th day after the last injection did not influence the lysin content. Believing that this was due to the fact that the tissue cells were no longer properly sensitized, another injection of sheep cells was given. Eleven days after this the titer was still at the same low level. An injection of foreign protein (typhoid vaccine) was then given, and an immediate marked liberation of lysins occurred. These antibodies disappeared rapidly. A second injection of typhoid vaccine in 7 days had no effect on raising the lysin titer, but the curve continued to fall rapidly. A third injection of red cells was then given and this time acted as a perfect antigen. The lysin content rose to its height in 10 days and remained high for several days. After it began to decrease another injection of red cells was given, but this time an injection of ascitic fluid was given only 4 days

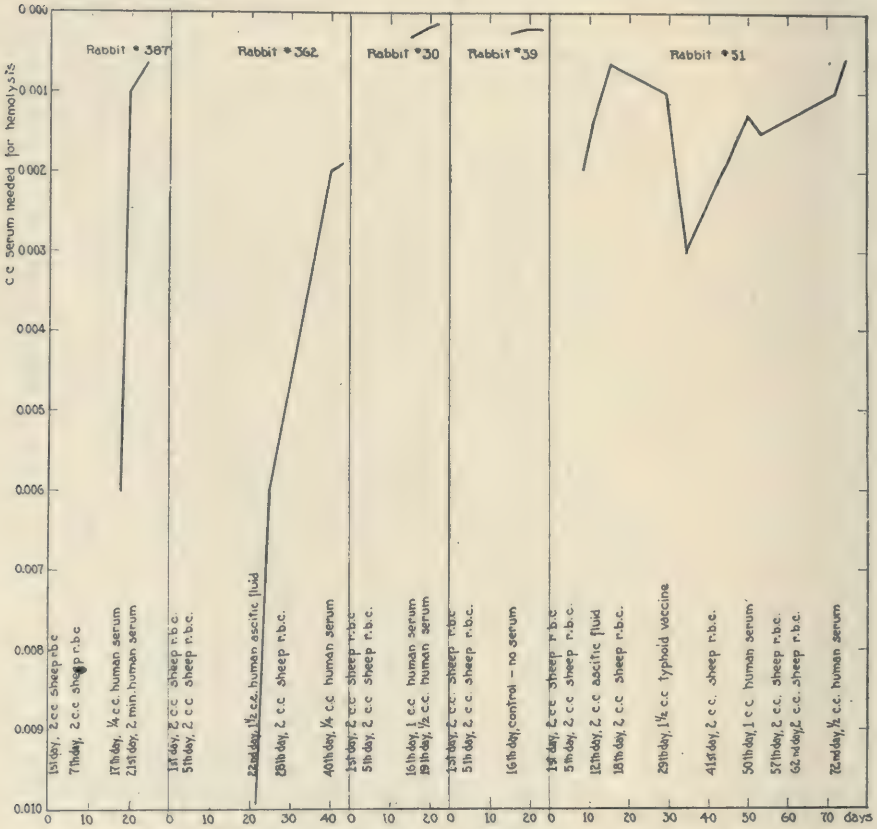

Chart 5 (Series 5).—Lysins for sheep cells.

later, in order to determine whether an early stimulation by foreign protein would serve to liberate antibodies. A very pronounced mobilization of lysins followed.

This alternating introduction of red cells and foreign protein was continued. Every time the mere injection of antigen (red cells) failed to produce lysins in the rabbit serum a mobilization of these antibodies could be effected by foreign protein. When, on the other hand, there was a normal response to the antigen, the foreign protein was followed by no further liberation of lysins. After the last 2 injections of red cells apparently all the receptors were not spontaneously liberated because there was a slight response to foreign protein 10 days later.

Rabbits 387 and 362 were of the same type, as they failed to respond to injections of red cells by a proper production of antibodies, but when then injected with foreign protein a marked liberation of lysins occurred.

Rabbits 30 and 39 responded very well to the antigenic stimulation of sheep cells; Rabbit 39 was used as a control while Rabbit 30 received human serum on the 11th day following the last injection of antigen. No marked difference was noted in the reactions of the 2 rabbits. This was observed also in a number of other cases. When the titer is already high, foreign protein will not bring about a further rise in antibody content.

Rabbit 51 represents a negative result which cannot be readily explained. The typhoid vaccine injected on the 29th day should have been followed by a rise in antibodies, according to our other observations, since the red cells 11 days earlier had not produced a liberation of lysins. In other respects, however, this curve is in accord with the general results.

## SUMMARY

In rabbits sensitized with streptococci a definite liberation of specific opsonins and agglutinins follows the injection of foreign protein.

A similar rise in specific opsonins also occurs in rabbits sensitized with meningococci.

Foreign protein injections have no effect on antibodies in typhoid immune rabbits.

In suitable rabbits, which do not readily produce lysins against sheep corpuscles, the injection of foreign protein within 10 days after the injection of antigen is followed by a marked liberation of specific lysins.

A variety of foreign proteins can be used. Human serum, typhoid vaccine, human ascitic fluid, and guinea-pig serum proved equally efficacious.

## CONCLUSION

The intravenous injection of foreign protein serves as a stimulus for the liberation of specific antibodies in animals in which the previously injected antigen is unable to cause such a liberation. This insufficiency may lie either in the antigen or in the rabbit.

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# RENAL FUNCTION IN EXPERIMENTAL HYDRONEPHROSIS.

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PLATES 17 TO 21.

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The object of this investigation is the study of the function of kidneys in the early stages of experimental hydronephrosis by means of the phenolsulfonephthalein test of Rowntree and Geraghty (1). The anatomic changes in hydronephrosis have been thoroughly studied by other observers and will be discussed here only as they pertain to the main purpose of the paper.

## *Anatomic Changes in Hydronephrosis.*

The anatomic changes after complete obstruction of the ureter will be discussed in stages corresponding in duration to the experiments given below.

During the first 3 days only slight changes are noted. Suzuki (2) found a considerable dilatation of the collecting and distal convoluted tubules, but only a slight dilatation of the proximal convoluted tubules at this stage. Fabian (3) noted a slight general dilatation of the tubules as early as 3 hours after ligature of the ureter. In a kidney with hydronephrosis of 3 days' duration I noted a considerable dilatation of the collecting tubules and those portions of Henle's loops lined by flattened epithelium, in both cortex and medulla. The proximal convoluted tubules, however, showed no definite change. The renal pelvis was moderately dilated.

In hydronephrosis of about a week's duration much more pronounced changes develop. Suzuki, in a hydronephrosis of 9 days' duration, found marked dilatation of the capsular spaces and the collecting tubules, and moderate dilatation of the distal convoluted tubules and both limbs of Henle's loops. The proximal convoluted tubules showed slight or no dilatation. A few casts were found distal to the first convoluted tubule. Casts in the medulla are mentioned by Ponfick (4). Scott (5) found collapse of some tubules in the apical portion of the pyramid, but my observations do not confirm this. In a hydronephrosis of 7 days' duration I observed marked dilation of the ureter and pelvis, and the kidney was distinctly pale. The cortex was thinner in the portions lateral to the sinus than elsewhere. The collecting tubules everywhere are dilated (Fig. 3). The proximal convoluted tubules are unchanged or slightly reduced in size in all parts of the kidney except in the portions lateral to the sinus where they are, for the most part, markedly reduced in size. Numerous casts are found in the collecting tubules in some parts of the kidney.

In hydronephrosis of about 2 weeks' duration the changes have become very pronounced. Suzuki described a narrowing of the parenchyma, most marked in the portions adjacent to the hilus. There was dilatation of the collecting and the distal convoluted tubules. The proximal convoluted tubules and the loops of Henle were usually collapsed, showing beginning atrophy. The atrophy was much more pronounced in the portions adjacent to the hilus than in the mid-sagittal portions. Occasionally a proximal convoluted tubule showed dilatation with a thinned epithelium. Very few casts were noted. In my control animal with a hydronephrosis of 2 weeks' duration the kidney was very pale. The pelvis and ureter were markedly dilated. The parenchyma was thinned, especially in the parts lateral to the sinus. The collecting tubules were all considerably dilated. The proximal convoluted tubules usually showed collapse with a moderate amount of atrophy. Some of these tubules were moderately dilated and had a thin epithelium (Fig. 8). In the portions lateral to the sinus of the kidney the dilatation of the tubules was pronounced and the atrophy of the proximal convoluted tubules was also much greater than in other portions of the kidney. Very few casts were to be seen (Fig. 9).

My observations agree with those of Suzuki that the portions of renal parenchyma lateral to the sinus of the kidney are much more severely injured than other parts of the kidney. The collecting tubules draining these lateral portions of the kidney are entirely collapsed. Suzuki's explanation of this phenomenon seems entirely satisfactory. He points out that the collecting tubules draining these portions of the kidney are subjected to much greater pressure than in other parts of the parenchyma.

In hydronephrosis of about 3 weeks' duration Suzuki noted marked atrophy of the proximal convoluted tubules and dilatation of the collecting tubules. The atrophic changes were much more pronounced in the lateral than in the mesial parts of the renal parenchyma. My observations on a hydronephrosis of 19 days' duration are essentially the same as Suzuki's. In the lateral portions of the kidney the proximal convoluted tubules are in a state of advanced atrophy with relative or absolute increase of the intervening connective tissue. The atrophy of the tubules in the mesial portions of the kidney is not nearly so pronounced. Collecting tubules are everywhere dilated. Only a few casts are to be seen.

Some authors mention areas of lymphocytic infiltration in hydronephrotic kidneys but these were not found in my experimental animals except where tubular atrophy was extreme. Probably most of these lymphocytic infiltrations mentioned by various authors are examples of spontaneous nephritis which is common in rabbits.

## *Anatomic Changes in Temporary Hydronephrosis with Subsequent Drainage.*

The investigators mentioned above studied the changes in hydronephrosis without drainage. Others removed the obstruction to the ureter after suitable intervals, established drainage, and studied the subsequent changes.

Rautenberg (6) ligatured the ureter close to the bladder to produce a hydronephrosis. At a second operation an anastomosis was made connecting the dilated ureter and the bladder, and at a third operation the sound kidney was removed. In this manner the changes which were produced in the period of obstruction plus those occurring in the period of drainage could be studied. The duration of hydronephrosis in his experiments varied from 2 to 6 weeks with subsequent drainage even as long as 6 months. He summarized his results as follows: Temporary ligature causes atrophy of the epithelial cells, especially in the convoluted tubules of the cortex. The degree of atrophy varies directly with the duration of obstruction and the injury is not uniformly distributed through the kidney. New connective tissue fills the space left by shrinkage of the tubules.

Some of the atrophic tubules return to normal size with a corresponding decrease of the new connective tissue. The extent of the recovery varies directly with the length of the drainage period. The recovery varies markedly in distribution and degree, sharply contrasted areas of atrophic and normal tubules being found in close association. He believes that the recovery is not permanent. The epithelial cells do not functionate normally, regressive changes again prevail, and eventually complete atrophy of the entire tubule results. The finding of increased blood vessel anastomoses between the kidney and surrounding tissue, claimed by Lindemann (7), was not confirmed by Rautenberg.

Bradford (8) ligatured the ureter on one side in dogs, and after intervals of from 11 to 40 days brought the dilated ureter to the abdominal surface and established a urinary fistula. The animals were kept for 7 to 51 days following the second operation. In three of the experiments a pyonephrosis developed, but the other nine remained free from infection. She found that after the second operation the kidney returned to its normal shape but was reduced to a third or half of its normal size. The ureter remained thickened and dilated. Microscopically therewas no general increase of connective tissue, except along the blood vessels. The renal tubules were crowded together, many had disappeared, especially in the cortex, and the epithelial cells were much smaller, with a distinct loss of granulation. These factors accounted for the decrease in the size of the kidney.

The changes in temporary hydronephrosis have been described also by Corbett (9) and by Amos (10).

## *Functional Studies in Kidneys with Hydronephrosis.*

The studies made of the functional capacity of kidneys with hydronephrosis may be divided into two groups. In the first group hydronephrosis was produced by a complete, sudden blocking of the ureter and functional studies were made at the completion of the desired period of dilatation. In addition, some of the experimenters of this group removed the obstruction, established drainage, and were able to make functional studies during the period of drainage. In the second group hydronephrosis was produced by a constant partial obstruction, sufficient to impede but not to stop the flow of urine.

*Hydronephrosis Produced by Complete Obstruction without Drainage.*—Suzuki studied the function of hydronephrotic kidneys by determining their ability to excrete indigo carmine. The dye was injected shortly before the animals were killed. Judging by the presence of carmine masses in the lumina and carmine granules in the cells of the tubules, he concludes that there is a progressive decrease of function with the duration of the hydronephrosis. In early stages the function is fairly good, but after 3 weeks it is greatly reduced and is apparently limited to a few convoluted tubules in the medial part of the kidney.

Boetzel (11) produced a unilateral hydronephrosis by ligaturing the ureter close to the bladder, then injected toluidine for functional study, and killed the animals 7 hours following the injection. Toluidine is eliminated by the convoluted tubules. After a dilatation of 6 days' duration no coloring matter was found in the pelvic fluid. After 21 days scarcely any granules, and after 30 days no dye granules were found in the renal cells. If the pressure is released in from 21 to 30 days the kidney excretes some of the dye in the urine, and after some time granules appear in the tubules. From this he concludes that accumulation of dye granules in the cells and excretion are independent, and that in Suzuki's experiments the carmine casts found are due to an abnormal secretion of tubules injured by the carmine itself. He agrees with Rautenberg that kidneys dilated 4 weeks or longer are injured beyond functional power and does not think that all the compressed tubules can recuperate.

Lindemann (12) found an impaired output of indigo carmine from a kidney the ureter of which had been ligatured for only 1½ hours.

Pfaundler (13) obstructed the ureter on one side for periods varying from 15 minutes to 6 hours and analyzed the fluid obtained. He compared the urine from both kidneys and found an increased volume of urine but a decreased concentration of urea and sodium chloride on the obstructed side.

Bainbridge (14) made a unilateral hydronephrosis in cats by ligaturing the ureter. After varying periods of dilatation he measured the pressure of the retained fluid and then analyzed it. After emptying the pelvis he collected the secretion from the two sides simultaneously, using salt as a diuretic. He found a steady diminution in the amount of water and solids excreted by the dilated side, decreasing directly with the duration of the hydronephrosis. The acidity was also diminished. The power of excretion was not entirely lost even when the hydronephrosis had lasted 2 months.

Heidenhain (15) tied one ureter and injected indigo carmine 24 hours later. The animal was killed as soon as the dye appeared in the urine from the normal kidney. The obstructed kidney differed from the normal in showing less color grossly and in the absence of dye granules in the cells of the convoluted tubules. Both kidneys showed carmine in the lumina of the tubules.

Kawasoye (16) ligatured the ureter on one side in nine rabbits and produced hydronephrosis varying from a few hours' to 9 months' duration. 20 minutes before killing the animals he injected indigo carmine. In the shorter periods of dilatation, up to 24 hours, he found a decreased amount of the dye in the tubular epithelium, but after 48 hours no dye whatever was found.

*Hydronephrosis Produced by Complete Obstruction with Subsequent Drainage.*—The studies given above were limited to a determination of the functional capacity of kidneys at the conclusion of a period of dilatation. Other observers drained the dilated kidneys and made subsequent functional observations. Rautenberg, in the series of experiments given previously, drained the dilated kidney into the bladder after desired periods of dilatation, and at a still later date removed the normal kidney. Seven rabbits with a preliminary hydronephrosis of 42 days and an interval of drainage of from 34 to 155 days died of renal insufficiency a few days after removal of the normal kidney. A similar result was obtained with two rabbits in which the duration of hydronephrosis was 28 and 29 days respectively. However, in a series of three rabbits with hydronephrosis of 3 weeks' duration and a period of drainage of from 22 to 31 days before removal of the normal kidney, two were still living a year later. The third rabbit died of a pyelonephritis. Albumin in small amounts and casts were found in the urine of both the living animals even after a year's time. In a second series of three rabbits with a shorter period of dilatation (about 2 weeks) and nephrectomy after a drainage period of from 14 to 19 days, two died. One was living 39 days after the nephrectomy but it was weak and emaciated. He concludes from these results that there is great individual variation in rabbits. The only functional test which Rautenberg applied was the ability to maintain life and this was lost in kidneys obstructed longer than 3 weeks. That there was still some unhealed renal lesion was shown by the presence of albumin and casts in the urine a year after the removal of the normal kidney.

Kawasoye determined the functional capacity with indigo carmine in a series of seven rabbits in which the obstruction was removed after varying intervals of dilatation. He found a complete restoration of function after a 4 day period and an incomplete restoration after a 7 to 14 day period. No restoration of function whatever, as shown by indigo carmine, was found in kidneys obstructed 21 days.

Corbett tied a ligature around the ureter just tight enough to produce complete obstruction without cutting through the tissues. After desired intervals the abdomen was again opened and the ligature loosened, allowing the urine to flow into the bladder. The normal kidney was removed at the same operation. The functional capacity of the injured kidney was then determined by a study of the total nitrogen and chlorides in the urine. He concludes that kidneys obstructed longer than 10 days do not excrete these substances in normal amounts.

Sollmann, Williams, and Briggs (17) produced a unilateral hydronephrosis of 107 days' duration and then made a urinary fistula for the dilated kidney. During 78 days of drainage no urine was excreted and only two drops were found in the shrunken kidney at autopsy.

*Hydronephrosis Produced by a Partial Obstruction.*—Keith and Snowden (18) tied a rubber band around one ureter to produce partial obstruction. The opposite kidney was removed at the same time. A hydronephrosis resulted in all cases with the fluid under a pressure of from 12 to 30 cm. of water as measured at autopsy. They noted a polyuria, a low specific gravity, and a trace of albumin, in the urine continuously after the operation. The phlorhizin test showed a moderate delay. The blood nitrogen showed a sharp rise at first and then maintained a fairly constant level to within a few days of death, when there was a great increase. The phthalein test was very sensitive and showed a slowing of excretion in the early stages, and in the later stages a progressive reduction in the total excretion when the rise in blood nitrogen occurred. The development of a pyelonephritis is only a question of time in animals operated on by this method.

Hermann (19), Lépine and Porteret (20), and Lindemann determined the effect of a constant back pressure on the kidney secretion by analyzing the urine. In addition to chemical analysis Schwarz (21), Cushny (22), and Filehne and Ruschhaupt (23) noted the effects of diuretics. Similar work on the effect of back pressure on kidney secretion was done by Brodie and Cullis (24) in decerebrated dogs. All are agreed that the flow of urine decreases with high and increases with a low back pressure. With the exception of Brodie and Cullis they found a decreased concentration of solid constituents. For a review of the literature on this phase of the question the reader is referred to Keith and Snowden.

In a recent article Keith and Pulford (25) describe the results obtained in experiments in which bilateral partial obstruction of the ureters was produced by ligaturing with rubber bands. The degree of obstruction obtained was not the same in all the experiments. In five animals definite functional impairment was shown after obstruction lasting about a week and the bands were then removed. Normal function returned in a few days. In another experiment the animal died shortly after removal of the obstruction, but in this case a pyelonephritis complicated the picture. Removal of the bands after 143 days of obstruction, in one experiment, failed to improve the impaired renal function, but observations were continued for only a week.

## EXPERIMENTAL.

### *Method.*

The animals used in the experiments were male rabbits, and all experiments were performed under ether anesthesia. To eliminate all animals in which an already existing renal lesion was present a careful urinalysis and a preliminary phthalein test were made. All rabbits showing any abnormalities in the urine or a phthalein output of less than 65 per cent for 2 hours were discarded. The average phthalein output in the rabbits used was higher than 65 per cent.

The rabbits were operated on in three stages. The preliminary operation consisted in the division and suturing of the ureter in the bladder wall. With all necessary aseptic surgical precautions and under ether anesthesia a median suprapubic incision about 4 cm. long was made exposing the bladder and lower portion of the ureter. The right ureter was then freed at its lower end close to the bladder, care being taken not to injure the blood vessels, ligatured doubly, and sectioned between. An incision about 2 cm. long was then made in the posterior wall of the bladder through the peritoneal and muscular coats down to the mucosa. The mucosa was separated from the muscular coat for a short distance on both sides and the ligatured ureter was then buried between the mucosa and the muscular coat by suturing the edges of the incision together. The abdomen was then closed.

At the second operation, after varying intervals of time, depending on the duration of hydronephrosis desired, the abdomen was again opened, under ether anesthesia, by an incision close to the first incision and parallel to it. An incision was made through the anterior wall of the bladder. The dilated end of the ureter, buried beneath the mucosa at the previous operation, was clearly visible as an elevation. The mucosa of the bladder over the elevated area was incised and the end of the ureter brought through and opened. The fluid from the dilated ureter then gushed forth into the bladder and drainage was established. The anterior wall of the bladder and abdomen were then sutured.

At the third operation, usually about 1 month after the establishment of drainage, under ether anesthesia, a nephrectomy through a dorsal incision was made on the left side, leaving the hydronephrotic kidney to perform the function alone. The animals were then kept in metabolism cages and the quantity of daily urine was measured. The urine was examined at frequent intervals. A record was kept of the weight. As often as it was deemed necessary phthalein tests were made. When the test returned to normal the animals were killed and the kidneys studied grossly and microscopically. The tissues were fixed in Helly's fluid, embedded in paraffin, and stained with hematoxylin and eosin.

A great many of the experiments were not successful. In a few cases infection from the operation, especially when it was necessary to operate at short intervals, resulted. Sometimes when the abdomen was opened for the second operation it was found that the ligature had slipped off and no hydronephrosis had resulted. Epidemics in the animal house caused the death of many of the experimental animals. In the series reported only those rabbits are included in which it was absolutely certain that the obstruction of the ureter was complete for the length of time indicated.

In order to determine the changes which occurred in the kidneys during the period of drainage, the ureter was ligatured in a number of control rabbits with normal urinary findings. These animals were killed after periods of dilatation corresponding to the duration of the hydronephrosis before drainage in the above experiments. By comparison the extent of recovery during the drainage period was determined.

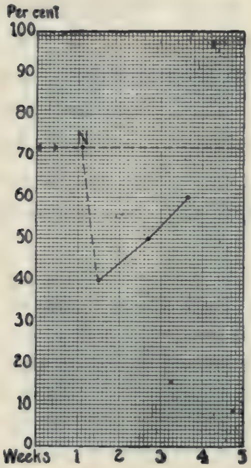
The phthalein test was made by injecting 1 cc. of the dye into the lumbar muscles. 2 hours later, with aseptic precautions, a No. 10 French, soft rubber catheter was introduced into the bladder. Catheterization is easily performed on male rabbits. The kidney and ureter were then gently pressed to expel as much urine as possible and the bladder was washed out with sterile salt solution. The reading was made in the usual manner in an Autenrieth-Königsberger colorimeter.

*Experiment 1. Complete Obstruction of the Ureter for 3 Days.*—Male rabbit. Preliminary phthalein 72 per cent. Urine normal. Right ureter ligatured. 3 days later, at the second operation when the ureter was opened, a smoky, brownish fluid gushed into the bladder. 5 days after the second operation the left kidney was removed. The phthalein test was 40 per cent on the 2nd, 50 per cent on the 10th, and 60 per cent on the 17th day after the nephrectomy (Text-fig. 1). A moderate amount of albumin and a few erythrocytes were present for a few days after the nephrectomy, but later the urinary findings were normal. The rabbit died 23 days after the nephrectomy from intestinal obstruction.

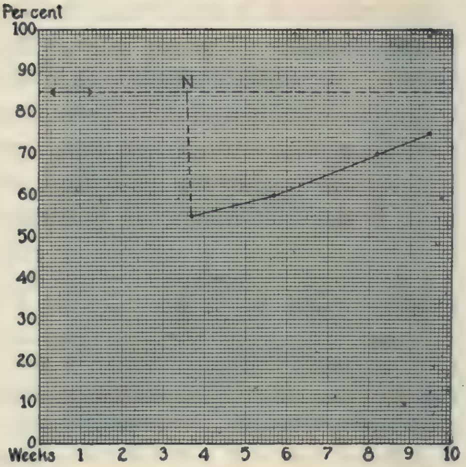
The right (hydronephrotic) kidney weighs 6.3 gm. The pelvis and ureter are slightly dilated. Fluid from the pelvis at autopsy shows numerous hyaline and granular casts and one cast of a renal tubule. The lining surface of the pelvis is smooth and shiny. No pus is present. The fluid in the bladder shows a small amount of pus but there is no marked congestion of the bladder wall. The only important difference between the right and left kidneys is the slight dilation of the right pelvis with a corresponding slight thinning of the renal parenchyma anteriorly and posteriorly. Microscopically no important differences are seen.

*Experiment 2. Complete Obstruction of the Ureter for 7 Days.*—Male rabbit. Preliminary phthalein test 85 per cent. Urine normal. Right ureter ligatured. 7 days later at the second operation the ureter was found well dilated, about the size of a small goose quill. When it was opened a thin, brownish fluid gushed out under tension into the bladder. Loss of weight between first and second operations 150 gm. 16 days after the second operation left nephrectomy was performed. The phthalein test rose gradually from 55 per cent the day following nephrectomy to 75 per cent 40 days later when the animal was chloroformed (Text-fig. 2). The kidney function had returned to the average normal although not so high as the original test. The rabbit had increased in weight 280 gm. during the experiment. The urine showed a faint trace of albumin continuously after the nephrectomy but there were no casts, erythrocytes, or pus cells.

The right kidney (hydronephrotic) weighs 6.6 gm. Grossly it shows several linear scars on each side of the pelvis radiating out from the hilus as a center, gradually becoming less distinct and disappearing about two-thirds of the distance to the convex border. They do not cross the rounded convex border. On section these linear scars are seen to correspond to small lateral evaginations of the pelvis. The cortical tissue in the linear areas is atrophic, apparently a permanent destruction of the renal tissue due to the hydronephrosis. The cortex shows a uniform thickness of about 4 mm. The microscopic structure of the renal parenchyma is practically normal (Fig. 1) except in the linear scars mentioned above which show atrophy of tubules, small collections of lymphocytes, and increase of connective tissue (Fig. 2). Fig. 3 shows a kidney obstructed by ligature of the ureter for 7 days, and, in all probability, represents the condition of the right kidney of this experiment at the time when drainage was established.



TEXT-FIG. 1. Function of a kidney obstructed for 3 days (Experiment 1).



TEXT-FIG. 2. Function of a kidney obstructed for 7 days (Experiment 2).

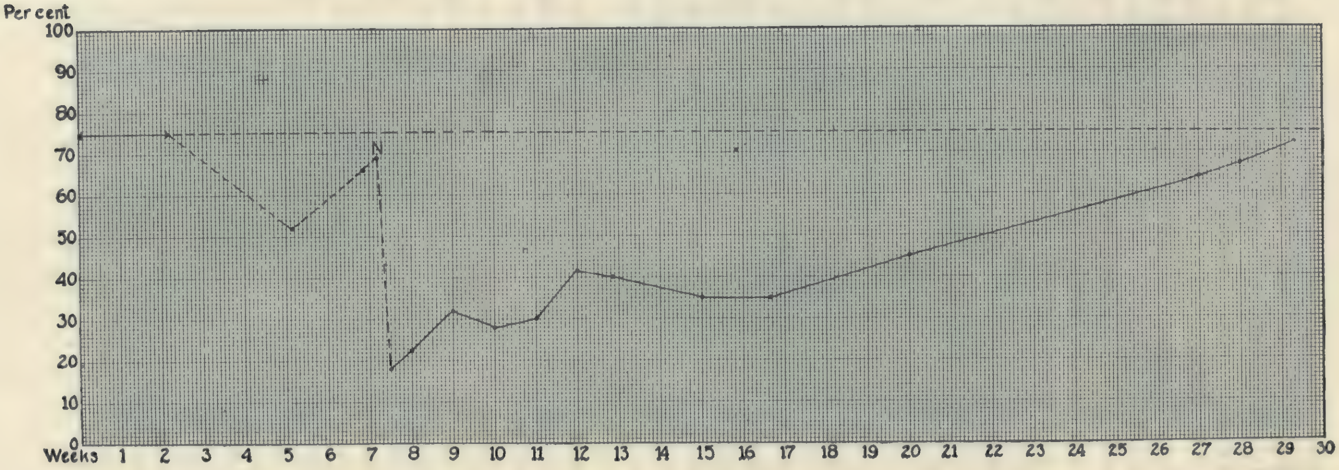
The ordinates indicate percentage of phthalein, and the abscissæ the time measured in weeks. The horizontal dotted line indicates the level of the preliminary phthalein test. The solid portion of this line indicates the period during which the ureter was tied off. N indicates the point at which the normal kidney was removed, and the phthalein readings are indicated by dots on the charted line.

*Experiment 3. Complete Obstruction of the Ureter for 14 Days.*—Male rabbit. Preliminary phthalein test 75 per cent. Urine normal. Right ureter ligatured. 14 days later at the second operation the ureter was found well dilated, about the size of a large goose quill. Brownish fluid gushed out under pressure when it was opened. The phthalein test was 52 per cent 21 days after the second operation, and 66 per cent 30 days after, which was the combined function of the normal and the hydronephrotic kidneys. 35 days after the second operation left nephrectomy was performed. A few erythrocytes, leukocytes, and hyaline and granular casts appeared in the urine for a few days. The phthalein test rose gradually from 18 per cent 2 days after the nephrectomy to 72 per cent 152 days later, when the animal was chloroformed (Text-fig. 3). The phthalein test had returned to within 3 per cent of the original test. The rabbit was active, well nourished, and normal except for a very faint trace of albumin which had been continuously present in the urine since the nephrectomy.

The right kidney (hydronephrotic) weighs 6.8 gm. It presents striking changes grossly. On both the anterior and posterior aspects of the kidney the mesial portion which forms the wall of the sinus is atrophic and depressed, not unlike an old infarct, while the lateral portion along the rounded border is hypertrophied and elevated. The two portions are sharply marked off from each other along a definite line, somewhat irregular at the limits of the pelvis. On section the parenchyma forming the lateral walls of the sinus is very thin and atrophic, apparently a permanent lesion in this region due to the hydronephrosis. The parenchyma along the convex border is hypertrophied, the cortex being 6 mm. in thickness, apparently a compensatory change. The pelvis is dilated and the ureter seems to be the same size as at the second operation 7 months previously.

Microscopic sections show practically normal structure along the convexity of the kidney. Anatomic recovery in this portion is apparently complete (Figs. 4 and 5). A few mitotic figures are seen. There is no increase of connective tissue except in a few small areas. But there is a sudden transition to atrophic tissue where the convex portion joins the flattened lateral walls of the sinus as described in the gross specimen. These lateral portions consist of numerous lymphocytes, markedly atrophic tubules, dilated capsular spaces with atrophic glomeruli, and diffusely distributed fibrous tissue (Figs. 6 and 7). Fig. 8 shows the mesial and Fig. 9 the lateral portion of a kidney obstructed for 14 days, and probably represent the condition of the corresponding portions of the right kidney of Experiment 3 at the time when drainage was established.

*Experiment 4. Complete Obstruction of the Ureter for 17 Days.*—Male rabbit. Preliminary phthalein 70 per cent. Urine normal. Right ureter ligatured. 17 days later at the second operation the ureter was found well dilated, about the size of a large goose quill. A thick brownish fluid gushed out under tension when it was opened. Loss of weight was 200 gm. between the first and second operations. 14 days after the second operation left nephrectomy was performed. The phthalein test was 3.5 per cent on the 3rd day following nephrectomy. The daily



TEXT-FIG. 3. Function of a kidney obstructed for 14 days (Experiment 3).

output of urine increased gradually from 60 cc. on the day following nephrectomy to 250 cc. on the 6th day and remained at about this level. A large amount of albumin, a few erythrocytes, leukocytes, and hyaline and granular casts were constantly present in the urine. The rabbit was very weak and inactive during the entire period and was found dead 9 days after the nephrectomy. The cause of death was apparently renal insufficiency, as no lesions aside from the kidney changes were found at autopsy.

The right kidney (hydronephrotic) weighs 7 gm. There is no evidence of pyelitis or pyelonephritis either grossly or microscopically. The finer structure cannot be studied well because of postmortem autolysis. There are no marked differences between this kidney and one obstructed for the same period without drainage.

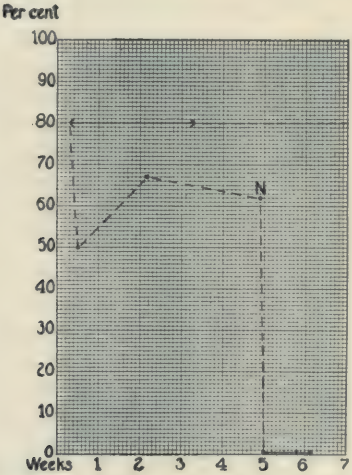
*Experiment 5. Complete Obstruction of the Ureter for 19 Days.*—Male rabbit. Preliminary phthalein 75 per cent. Urine normal. Right ureter ligatured. 19 days later a ureterocystostomy was performed as the preliminary operation was not the usual one but consisted of only ligature and section of the ureter. The ureter was dilated to the size of a large goose quill. A large amount of dark brownish fluid gushed out under tension. Loss of weight of 150 gm. between first and second operations. 18 days after the second operation the rabbit weighed 50 gm. more than at the beginning of the experiment and a left nephrectomy was performed. On the day following nephrectomy the phthalein test was 0. On the 2nd day 20 cc. of urine were excreted showing a tinge of phthalein, a moderate amount of albumin, but no erythrocytes, leukocytes, or casts. The rabbit became very weak and died 3 days after nephrectomy. The urine in the bladder at autopsy showed a minute trace of phthalein. There was no obstruction at the ureteral orifice in the bladder wall. No lesions aside from those in the kidney were found, and death was due apparently to renal insufficiency.

The right kidney (hydronephrotic) weighs 9.1 gm. The pelvis and ureter are markedly dilated. There is no evidence of infection. There are no important changes present, grossly or microscopically, other than those ordinarily found in a simple hydronephrosis of like duration.

*Experiment 6. Complete Obstruction of the Ureter for 19 Days.*—Male rabbit. Preliminary phthalein 70 per cent. Right ureter ligatured. 19 days later at the second operation the ureter was about ½ inch in diameter and filled with a brownish fluid under tension. 3 days after the second operation left nephrectomy was performed. The phthalein test was 3 per cent on the 2nd day following the nephrectomy. The daily output of urine increased from 40 cc. on the day following nephrectomy to 80 cc. on the 4th day. Urinalysis showed a trace of albumin, a few leukocytes, and a few erythrocytes. The rabbit became very weak and died on the 4th day. At autopsy, besides the renal changes, there was found a small, circumscribed, inspissated abscess in the lower lobe of the right lung. Apparently the cause of death was renal insufficiency, as the lung condition was well walled off and chronic.

The right kidney (hydronephrotic) weighs 12.8 gm. A few subcapsular petechiæ are present. The pelvis and ureter are markedly dilated. There is no evidence of infection. No striking microscopic differences between this kidney and one dilated for a similar period of time without drainage are to be noted.

*Experiment 7. Complete Obstruction of the Ureter for 21 Days.*—Male rabbit. Preliminary phthalein 80 per cent. Urine normal. Right ureter ligatured. The phthalein test was 50 per cent the day following, and 67 per cent on the 13th day after ligature of the ureter. 21 days after the first operation the ureter was found dilated to about ½ inch in diameter and a dark smoky brown fluid gushed out when the opening was made into the bladder. The phthalein was 62 per cent 10 days after the second operation, which was the combined function of the normal and the hydronephrotic kidneys. 11 days after the second operation left nephrectomy was performed. The phthalein test was 0 on the day following nephrectomy, but minute traces were present in the urine during the next few days. On the 5th day the phthalein test showed only a faint trace (Text-fig. 4). The amount of urine increased from 2 cc. on the day following the nephrectomy to 100 cc. on the 8th day. A large amount of albumin, with a few erythrocytes, leukocytes, and hyaline casts was continuously present. The rabbit was found dead on the 9th day apparently from renal insufficiency, as no other lesions were present at autopsy.



TEXT-FIG. 4. Function of a kidney obstructed for 21 days (Experiment 7).

The right kidney (hydronephrotic) weighs 11.6gm. The pelvis and ureter are markedly dilated. There is no evidence of infection. No marked changes other than those usually found in a simple hydronephrosis of 21 days' duration are present (Fig. 10). A kidney with such extensive changes is unable to function sufficiently to maintain life.

## DISCUSSION.

Hydronephrosis of 3 days' duration causes considerable dilatation of the collecting tubules and the portions of Henle's loop lined by flattened epithelium. No anatomic changes are recognizable in the convoluted tubules. The kidney of Experiment 1 returned to about normal function, as measured by the phthalein test, in 15 days. The only abnormality seen in the kidney after functional recovery is a slight dilatation of the renal pelvis and a slight thinning of the renal parenchyma on the lateral aspects of the renal sinus.

When hydronephrosis has been present for 7 days there is a marked dilatation of the pelvis with considerable thinning of the parenchyma laterally. The dilatation of the collecting tubules is more marked than in the 3 day stage and there is some reduction in size of many of the convoluted tubules, those in the portions lateral to the sinus being especially compressed. The kidney of Experiment 2 had an initial phthalein excretion of 55 per cent and returned to normal function in about 40 days. After functional recovery the kidney showed a dilated pelvis with thinning of the parenchyma laterally. The lateral portions of the kidney showed a few scars due to complete atrophy of small portions of the cortex. Elsewhere the anatomic picture was about normal.

A kidney with hydronephrosis of 14 days' duration shows more dilatation of the pelvis and ureter and greater thinning of the renal parenchyma, especially in the parts lateral to the sinus, than in the 7 day stage. The collecting tubules are considerably dilated and the proximal convoluted tubules are collapsed with some atrophy. The supporting connective tissue is increased. The lateral portions of the kidney show the maximum dilatation of the collecting tubules and maximum atrophy of the proximal convoluted tubules. The initial phthalein output of the kidney in Experiment 3 was 18 per cent, which returned to normal only after 152 days. The pelvis and ureter were still dilated after functional recovery. The portion of renal parenchyma forming the wall of the sinus was completely atrophied. As already stated, Suzuki's explanation of the greater injury sustained by this portion of the kidney seems satisfactory; *i.e.*, the collecting tubules draining this portion, owing to their longer and more indirect course to the papilla are subjected to greater pressure than those draining the central portion. The destruction of these collecting tubules results in atrophy of their convoluted tubules and glomeruli. The parenchyma along the convex border of the kidney was almost normal, the convoluted tubules having regained their normal size and appearance.

In the kidneys with hydronephrosis longer than 14 days (17, 19, 19, and 21 days) there is marked dilatation of the ureter and pelvis. The proximal convoluted tubules are in a state of advanced atrophy with relative or absolute increase of connective tissue. The collecting tubules are markedly dilated everywhere. No kidney of this group was able to secrete over 3.5 per cent of phthalein. No improvement in secretory power was obtained and no rabbit lived sufficiently long after drainage to develop any notable renal changes other than those seen in simple hydronephrosis of equal duration.

It is to be noted that the ureter and pelvis showed no tendency in any of the experiments to return to normal size. No evidence of secondary infection was seen in the pelvis, ureter, or kidney parenchyma.

If a nephrectomy is performed on a normal animal the remaining kidney excretes, a few days later, nearly as much phthalein as the previous output of both kidneys. The atrophy produced by hydronephrosis greatly reduces this ability until compensatory hypertrophic changes can occur. The kidney with hydronephrosis of 3 days' duration required 15 days to recover 20 per cent of function, or 1.33 per cent per day. The one with hydronephrosis of 7 days' duration required 40 days to recover 20 per cent, or 0.5 per cent per day. The one with hydronephrosis of 14 days' duration required 152 days to recover 54 per cent, or 0.35 per cent per day. It can be readily seen, therefore, that the longer the period of dilatation the slower is the rate of recovery after drainage. The delayed recovery of function in the more prolonged cases of hydronephrosis is apparently due to the greater degree of atrophy of the convoluted tubules. The more advanced the atrophy the slower seems to be the return to normal. The destruction of collecting tubules is not the cause of the atrophy of the convoluted tubules except in the lateral portions of the kidney.

In view of the slow recovery after complete hydronephrosis for 2 weeks it is not surprising that Keith and Pulford noted no appreciable improvement in a week in a hydronephrosis obtained by partial obstruction of the ureter for 143 days. The rapid recovery noted by them in other experiments after partial obstruction for about a week, when secondary pus infection did not complicate the picture, is also in accordance with the above results.

The contention of Rautenberg that the regeneration after drainage is not permanent and that eventually complete atrophy ensues is not borne out by my experiments. The presence of albumin in the urine in Experiment 3 even at the end of the experiment indicated renal disturbance; but the high phthalein excretion and the autopsy findings showed no evidence of a secondary atrophy.

Rabbits with hydronephrosis longer than 14 days did not live long after removal of the normal kidney. In this group the greatest phthalein output was 3.5 per cent. They all died apparently of renal insufficiency. One may conclude from these results that when a hydronephrotic kidney shows so low a phthalein excretion it will be unable to maintain the life of the individual. The question arises whether such a hydronephrotic kidney would improve sufficiently to maintain life if the normal kidney were not removed until 6 months or more after the establishment of drainage. My observations do not determine this point.

Another point, likewise not determined by the above experiments, is whether the hydronephrotic kidney would recover functionally as it did in Experiment 3 if the normal kidney had not been removed; *i.e.*, whether the stimulus of compulsory function is necessary for recovery.

In the above experiments kidneys obstructed longer than 14 days did not recover; but Rautenberg had two rabbits with hydronephrosis of 3 weeks' duration that lived a year after removal of the normal kidney. This probably means, as Rautenberg suggests, that there is considerable individual variation in rabbits.

## SUMMARY.

Complete obstruction of the ureter causes atrophy of the renal parenchyma, especially marked in the portions lateral to the renal sinus. The longer the duration of the obstruction, the greater is the degree of atrophy.

If the obstruction is removed within 2 weeks the kidney may regain its normal structure except for a varying amount of atrophy in the lateral portions.

Kidneys obstructed for 2 weeks or less may regain their normal function, as measured by the phthalein test.

The longer the period of obstruction, the slower is the rate of recovery. A hydronephrosis of 7 days' duration required 40 days, and one of 14 days' duration required 152 days to recover normal function.

I wish to express my indebtedness to Dr. E. T. Bell for criticisms and suggestions during the progress of this work.

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## EXPLANATION OF PLATES.

### PLATE 17.

FIG. 1. Cortex of the kidney of Experiment 2, showing complete recovery after obstruction of the ureter for 7 days.

FIG. 2. Section through one of the linear scars occurring in the kidney of Experiment 2.

### PLATE 18.

FIG. 3. Cortex of a control kidney with complete obstruction of the ureter for 7 days. Compare with FIG. 1.

FIG. 4. Cortex of the kidney of Experiment 3, from the convex border, showing almost complete recovery after obstruction of the ureter for 14 days.

### PLATE 19.

FIG. 5. High power view of FIG. 4.

FIG. 6. Cortex of the kidney of Experiment 3, from the lateral wall of the sinus of the kidney, showing extreme atrophy after obstruction of the ureter for 14 days.

## PLATE 20.

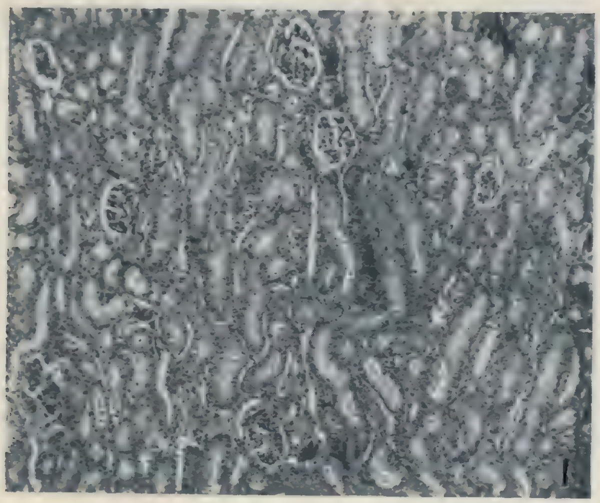
FIG. 7. High power view of Fig. 6.

FIG. 8. Cortex from the convex border of a control kidney with complete obstruction of the ureter for 14 days. This portion of the kidney recovers after drainage. Compare with FIG. 4.

## PLATE 21.

FIG. 9. Cortex from the lateral wall of the sinus of a control kidney with complete obstruction of the ureter for 14 days. This portion of the kidney does not recover after drainage. Compare with FIG. 6.

FIG. 10. Cortex of the kidney from Experiment 7 with complete obstruction of the ureter for 21 days.



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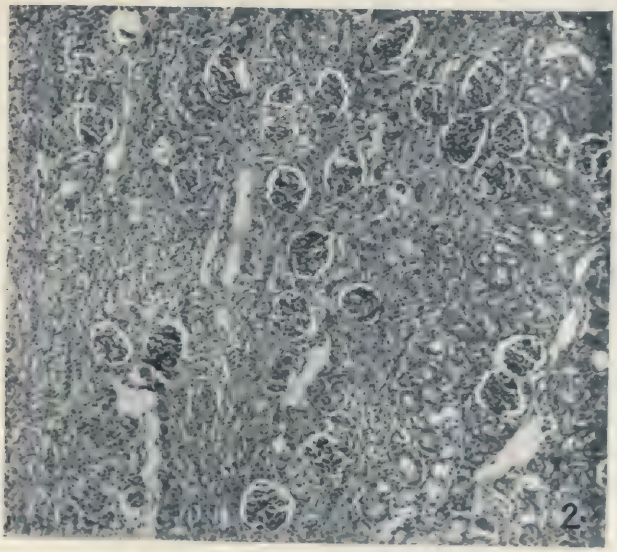

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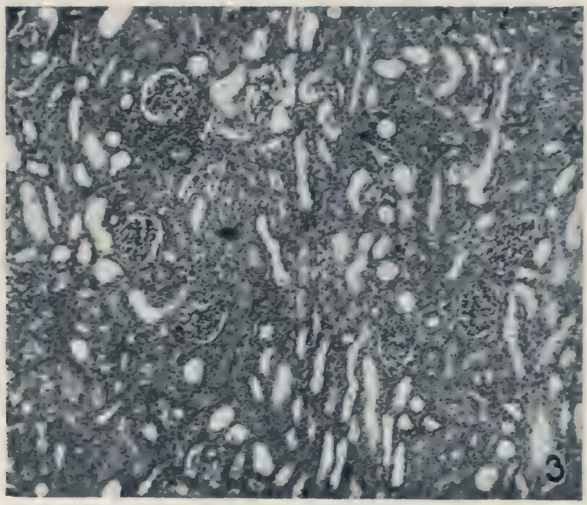
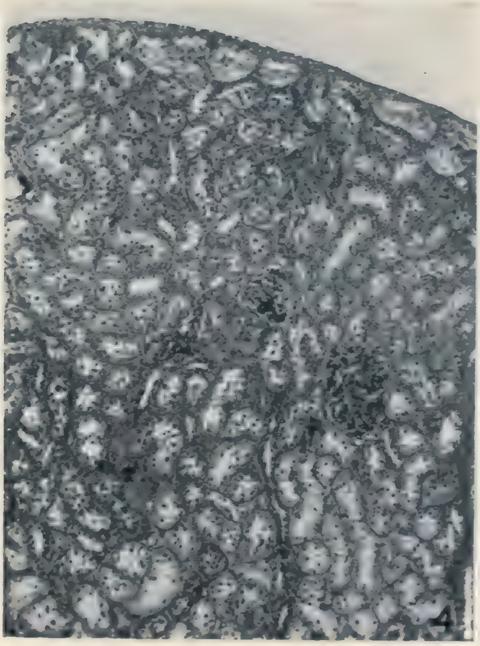
(Johnson: Renal function in hydronephrosis.)





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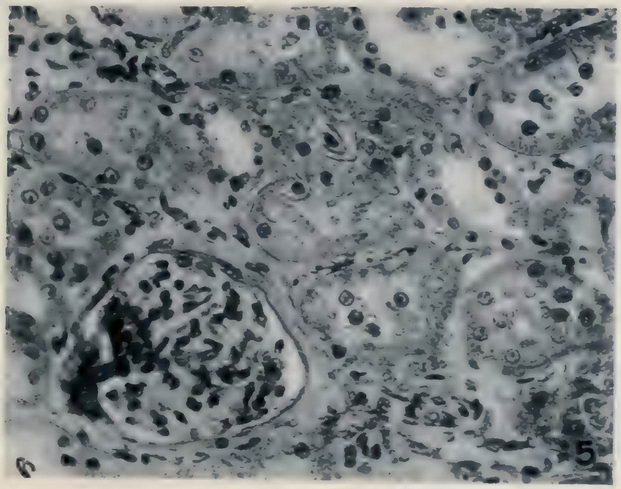

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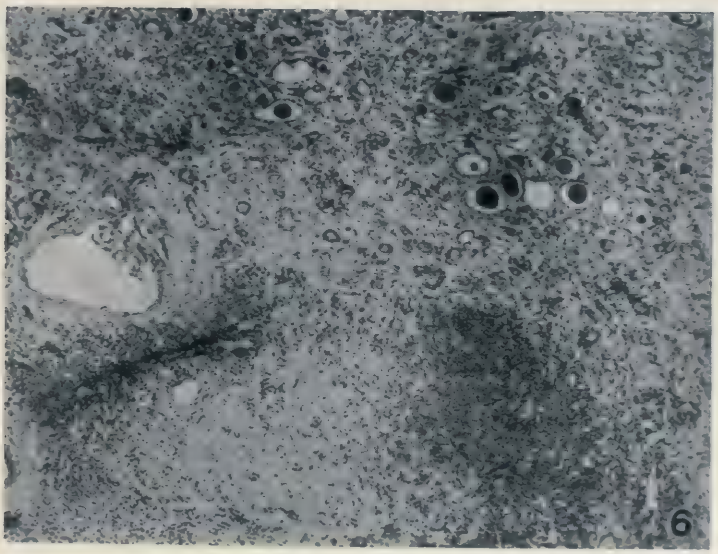

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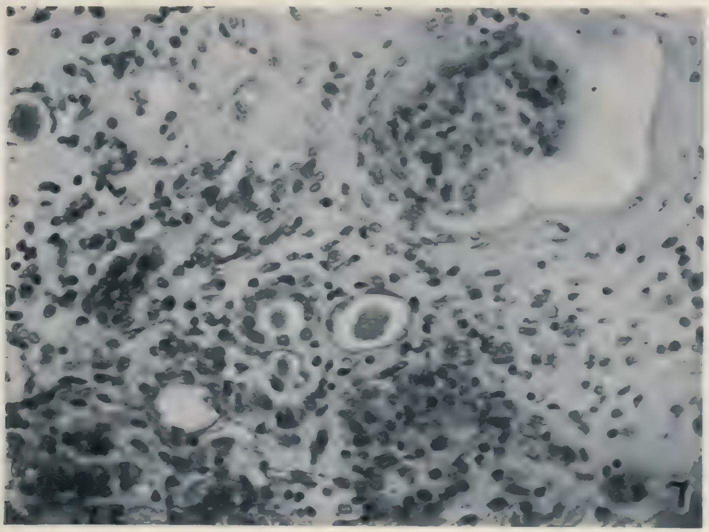
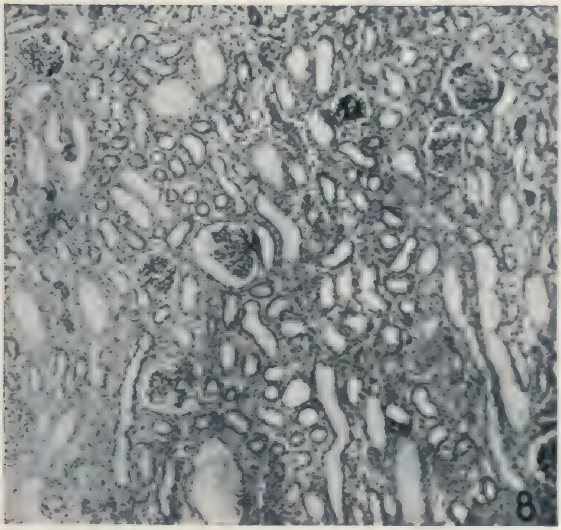
(Johnson: Renal function in hydronephrosis.)





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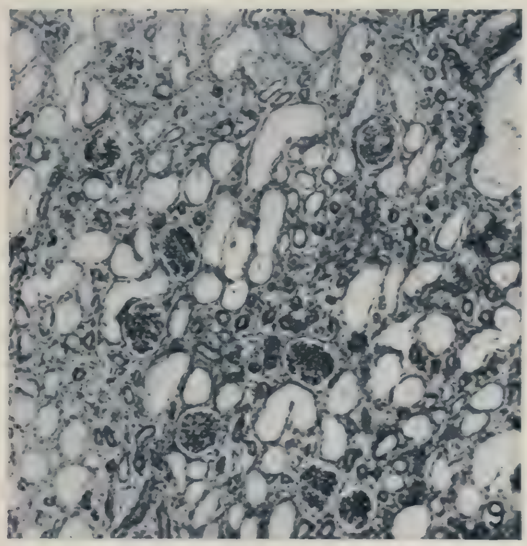

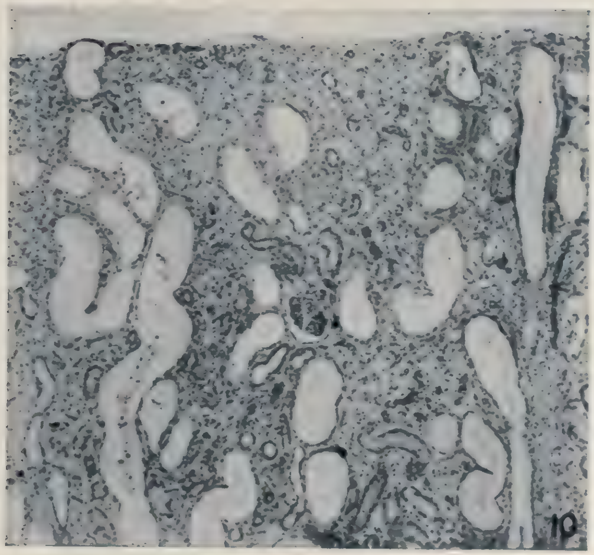

(Johnson: Renal function in hydronephrosis.)

# THE EFFECT OF HIGH PRESSURES ON BACTERIA

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# THE EFFECT OF HIGH PRESSURES ON BACTERIA

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The work recorded in this paper is the result of an attempt to study the mechanism of immunity and incidentally to test a theory regarding the failure of vaccine therapy as practiced at the present time.

During the past 8 or 10 years bacterial vaccines have been used extensively, but, on the whole, with very unsatisfactory results. Were it not for the excellent theoretical foundation on which vaccine therapy rests, its use would probably have been relegated to the past. Bacterial vaccines, as used today, judged by the practical results obtained, are of little or no value aside from typhoid vaccine as used prophylactically and the undisputed value of staphylococcic and B. acne vaccines, in certain types of cases.

Protective immunization experiments with killed cultures on laboratory animals have likewise not been crowned with marked success. Facts such as these may readily be gleaned from a survey of the literature. The conclusion would therefore seem justified that the dead organisms do not offer a suitable antigen for immunization processes.

During the past year while conducting some perfusion experiments on rabbits, we were much impressed by the rapidity with which bacteria were taken up by phagocytes. The particular experiment in question concerned the perfusion of the liver of rabbits with emulsions of staphylococci. By inserting cannulae in the portal vein and superior vena cava the liver could readily be perfused with Locke's fluid, containing large quantities of staphylococci in suspension. It was found that a fluid containing 9,000,000 organisms per cubic centimeter could be sterilized in a few minutes by being passed through this organ. The endothelial cells of the liver, on section, were found to be literally packed with bacteria following this operation. This observation suggested that vaccines injected into an individual for the purpose of

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immunization probably meet a similar fate. The phagocytes, whose function it is to seize any foreign material entering the body, failing to recognize the benevolent intention of the immunizer, probably take up the killed cultures with even more greed than the living bacteria. Metchnikoff[1] and his pupils have shown that the digestion of such incorporated particles by phagocytes may be very rapid, especially when it concerns dead bacteria.

It is a well recognized fact that an antigen which has undergone even partial digestion is no longer capable of stimulating antibody formation, and hence ceases to be an antigen. The natural deduction is therefore to assume that phagocytosis defeats, in a measure at least, attempts to immunize with killed bacterial cultures. Metchnikoff and his school would have us believe that the phagocytes are the source of antibody formation, but the evidence presented in support of this contention is far from conclusive.

If we now examine antigens, such as diphtheria and tetanus toxins, which are not in the form of particles, but in a diffused state and hence not so accessible to the phagocytes, there is little difficulty in attaining a high degree of immunity. An animal may likewise be given a few injections of foreign protein, such as egg white, with the result that a high titer of immune serum is formed. The exception to this rule would seem to be the immunization with tissue cells and red corpuscles in particular. A few injections of foreign red cells will usually give a serum with a very high lytic titer. This may, however, be explained by the fact that tissue cells are taken up by the so-called macrophages, which are large cells with a rigid nucleus, and which are slow to arrive on the field of action. It is not improbable that the antigenic principle is dissolved from the injected cells by the tissue fluids before phagocytosis takes place. Prompted by these considerations an attempt was made to extract the antigenic principle from bacteria in a form not so readily accessible to phagocytes in the hope that it might give vaccine therapy the much needed "raison d'être."

The conversion of bacteria from the solid or semisolid state to a state of diffusion is not an easy matter, owing to the firm outer membrane. Numerous investigators have subjected bacteria to various physical influences looking to this end, such as high pressure, shaking, freezing and grinding processes, but hitherto with little or no success.

E. Büchner[2] was the first to obtain definitely positive results by subjecting living cells to high pressure. By the use of a hydraulic press he was able to separate zymase from the yeast cell. Later, Hans Büchner,[3] by using the same apparatus, subjected bacteria to a pressure of 500 atmospheres in an attempt to isolate ferments and endotoxins, but with rather disappointing results. Certes[4] found that a pressure of 350 to 500 atmospheres had no effect on putrefactive processes. Yeast cells under a pressure of 400 atmospheres were still able to ferment sugar. The same investigator found that the pathogenicity of anthrax bacilli was not impaired after having been subjected to a pressure of 600 atmospheres. D'Arsonval[5] and Charrin on the other hand found that $CO_2$ under a pressure of 50 atmospheres destroyed B. pyocyaneus in 2 hours. Their work was repeated by Sabruzes[6] and Bazin, Schaeffer[7] and Freudenreich, and Krause,[8] all of whom failed to confirm the findings of the French investigators. In summing up the results of high pressures on bacteria Gottschlich[9] concludes that they have little or no effect.

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[1] Metchnikoff: L'Immunite, 1901

[2] Buchner, E.: Ber. d. deutsch. Chem. Ges., 1897, 30, 117.

In our work many pieces of apparatus have been constructed, but only those with which definite results were obtained will be described. We first studied the effect of a direct load on bacteria.

For this purpose a piece of nickel-chrome-steel was obtained from one of the large steel companies, which also kindly furnished us with an analysis of the material. A hole ⅞ inch in diameter was bored through a piece of steel 5 inches by 3½ inches, and a piston, likewise of nickel-chrome-steel, was ground to fit the cylinder. One end of the cylinder was closed by a steel wedge. The cylinder, piston, and wedge were then tempered to withstand a pressure of 225,000 lbs. per square inch. The bacteria that were to be subjected to pressure were mixed with infusorial earth, after which they were placed in a leaden cup about 2 inches long and of a bore to fit the steel cylinder above described. The leaden cup, about two-thirds filled, was placed in the cylinder in an inverted position and forced to the bottom of the cylinder where it rested on a leaden disk which, in turn, rested on a steel wedge closing one end of the cylinder. The piston was then inserted and the apparatus placed in a testing machine such as is used by engineers in testing the strength of materials. The particular machine in question was capable of lifting a load of 200,000 lbs. The tests were usually inaugurated at 6 p. m. and terminated 14 hours later.

The following organisms have been studied in this manner: B. typhosus, B. coli, B. tuberculosis, B. proteus, B. subtilis, staphylococci, streptococci and pneumococci.

The pressures used ranged from 3,000-12,000 atmospheres. On termination of each experiment cultures were made on dextrose broth and, whenever possible, plates were made before and after the experiment. The general statement may be made that a pressure of 3,000 atmospheres was not sufficient to destroy any of the bacteria studied,

[3] Buchner, E.: München. med. Wchnschr., 1897, 44, 299.

[4] Certes: Compt. rend. Acad. de Sc., 1889, 99, 385.

[5] d'Arsonval & Charrin: Compt. rend. Soc. Biol., 1893, 467, 764.

[6] Sabrazés & Bazin: Koch's Jahresb. f. Gärungsorganism, 1893, 34.

[7] Schaffer & Freudenreich: Ann. d. Micrographie, 1890, 4, 502.

[8] Krause: Centralblatt f. Bakt., etc., 1902, O., 31, 673.

[9] Gotschlich: Kolle-Wassermann Handb. d. path. Mik., 2 Aufl., 3, 460.

while a pressure of 6,000 atmospheres for 14 hours was found to destroy all non-spore-forming organisms. The spores of B. subtilis were not regularly killed at pressures of 12,000 atmospheres. The bacteria would undoubtedly have been destroyed by these pressures in less time than that used; further studies are being undertaken to determine this point, however.

Having thus determined that it was possible to destroy bacteria by physical pressure, the question naturally arose as to the mechanism operative in this procedure. It was probably not a question of expressing the cytoplasm from the organisms, as was attempted by Büchner, inasmuch as the pressure exerted on the bacteria was uniform from all sides. The small amount of entrapped oxygen in the container could scarcely be responsible for the phenomenon. It was also felt that the element of heat development could be ruled out without further consideration. Two other factors were still to be considered: the direct effect of the pressure, and its sudden release. Which of these two was the important factor was, by the very nature of the experiment, difficult to determine, but it was not improbable that both factors played an important rôle in killing the bacteria. The interesting observation was made that bacteria subjected to high pressure were difficult to stain; as a rule only shadows could be discerned where it concerned gram-negative organisms, while the gram-positive ones usually lost their specific staining reaction.

It would seem probable from these experiments that the death of the bacteria was due either directly to the pressure or its sudden release or to a possible combination of both.

In an effort to analyze more fully some of the problems which had presented themselves during the process of these investigations, studies were inaugurated to determine the effect on various bacteria of gases under high pressure, such as $CO_2$, H, and N. An apparatus was constructed for this purpose which would enable us to subject bacteria to various gas pressures and suddenly release it. The apparatus used for these experiments may briefly be described as follows:

A steel tube, 8 inches in length and 1½ inches in diameter, lined with block tin, was attached to the desired gas tank by a suitable coupling. The opposite end of the apparatus was fitted to a brass head containing a small receiving chamber. The bacteria to be studied were placed in a sterile test tube resting on a coiled spring in the bottom of the tin-lined steel tube. The receiving chamber was provided with a valve, connected with a glass tubing which extended to the bottom of the test tube containing the bacteria. The apparatus was then attached to the gas tank and left for the desired time. When the valve leading to the receiving chamber was opened, the pressure of the gas forced the bacterial suspension up the glass tube, through the valve into the receiving chamber. The fluid so driven into the receiving chamber could be collected from a small spout into a sterile test tube. It will thus be seen that the pressure was released suddenly as the fluid passed the valve. The valve was under such control as to permit the removal of the fluid drop by drop if desired. We are indebted to Mr. David Crowther, Mechanician and Instructor in Dental Mechanics, University of Minnesota, for valuable mechanical assistance. We are indebted also to Dr. J. F. McClendon for helpful suggestions.

It was found that $CO_2$ under a pressure of 50 atmospheres would destroy B. typhosus, B. coli, B. tuberculosis, B. pyocyaneus, staphylococci, streptococci, and pneumococci in a period of time ranging from 1½-2½ hours. It was interesting to observe that the gram-negative bacilli could be brought to a marked degree of disintegration, although disintegration of all the bacteria in suspension was never attained. The gram-positive cocci, on the other hand, suffered little morphologic change aside from slight irregularity in size, and often a tendency to lose their gram-positive character. The importance of suspending bacteria in distilled water instead of broth or physiologic salt solution was emphasized by the fact that when the latter were used very little disintegration of the organisms was noted. It was found that $CO_2$ of less than 40 atmospheres produced no effect whatever on the bacteria studied. Yeast cells were found to be unaffected by $CO_2$ after exposure to this gas for 48 hours. This may be due to the fact that yeast cells are normally active $CO_2$ producers, and their external membrane therefore probably offers little or no resistance to the $CO_2$ molecule. The osmotic equilibrium of these cells was probably not disturbed by the sudden change in the molecular concentration of the fluid. $CO_2$ likewise has no effect on the proteolytic ferments of B. pyocyaneus and B. proteus; these ferments were found to be as active after exposure to this gas at 67 atmospheres for 20 hours as were the controls kept at room temperature.

Having then found that bacteria could be destroyed by relatively low gas pressure, we were once more confronted with the problem as to the mechanism of the various physical and chemical forces involved in the experiment.

It seemed probable that the acidity of the $CO_2$ solution under the pressure used might be responsible for the killing of the organisms, although their disintegration was evidently due to the sudden expansion of the dissolved gas. Being unable to find any work dealing with the degree of acidity of $CO_2$ solutions under pressure, experiments to determine the H ion concentration of this gas under various pressures were undertaken. The methods suitable for this particular experiment were, owing to its peculiar nature, limited. The colorimetric method seemed to be the most feasible. For this experiment a steel tube 4 inches long and 1 inch in diameter was lined with pure block tin. The ends of the tube were closed with glass lenses, supported further by heavy steel burrs. The indicator to be used was then placed in this apparatus which by means of a suitable coupling was attached to the $CO_2$ tank. Any change in the indicator could thus readily be determined. In order to be able to detect even a slight change in the indicator it was found necessary to construct a duplicate apparatus always containing the same indicator which could be used as a control. The $P_H$ was found to be between 10-3 and 10-4, though probably nearer 4 than 3. It was found that the amount of pressure used had very little effect on the degree of acidity; in fact, experimentally, it was difficult to show any difference in the degree of acidity between 1 and 50 atmospheres' pressure, although theoretically the $P_H$ of $CO_2$ at 50 atmospheres is 10-3.15 and at 1 atmosphere 10-3.6. Our failure to establish any difference in the degree of acidity of this gas under various pressures was probably an insufficient number of indicators of the proper range were used.

The next logical step in this series of experiments seemed to be to determine the effect of this degree of H ion concentration on the bacteria studied. Hydrochloric acid, acetic and phosphoric acids were prepared to a H ion concentration of 10-3.15, the maximum theoretical acidity of the $CO_2$ used. It was found that B. coli and the staphylococcus would live from 7-10 days in the hydrochloric and acetic acid of this acidity. The phosphoric acid (which is not buffered appreciably by proteins) was found to be more injurious to the organisms. However, both the staphylococcus and B. coli survived the action of the phosphoric acid for 48 hours. Our findings in this respect do not coincide fully with those of Michaelis,[10] who found that B. coli was destroyed in 48 hours in a solution of lactic acid at a $P_H$ of 10-4. It is not improbable that various strains of the same organism may vary in regard to their resistance to acids.

These experiments seem to justify the conclusion that the acidity of the $CO_2$ is not an important factor in the destruction of the organism. It therefore seemed probable that the high molecular concentration or the sudden change in the osmotic tension of the fluid, or possibly both, were the factors which destroyed the bacteria. $CO_2$ is very soluble in water, 0.8 of a volume being soluble for each atmospheric pressure. The maximal $CO_2$ pressure used in our experiments was 67 atmospheres, which, according to the law of the solubility of gases, would cause 52 volumes of $CO_2$ to go into solution. In order to determine the effect of this molecular tension on bacteria a solution of NaCl of corresponding concentration (7.2%) was prepared. Staphylococci and B. coli were suspended in this solution, which was acidified to a point of $P_H$ 10-3.15, and cultures were taken hourly. It was found that these organisms were killed in about 15 hours. It does not follow, naturally, that, because the solution of $CO_2$ and sodium chlorid were of the same molecular concentration, their effect on bacteria would be identical. The effect of each on the membrane of the bacteria might be widely different; possibly the size of the molecule might be a vital factor, not to mention the possibility of the influence of incidental chemical reactions. Ignoring, for argument's sake, these unknown factors, and assuming the effect of the two solutions on bacteria to be much the same, we are led to the conclusion that the sudden release of the pressure, thereby lowering the osmotic tension of the fluid, is the vital factor in killing the bacteria, as it was found that bacteria were killed in from 1½-2½ hours in $CO_2$ solutions, whereas 15 hours were necessary to kill them in acidified salt solution of the same molecular tension. The 1½-2½ hours probably represents the time required for the gas to become dissolved in the water and to diffuse through the bacterial membranes.

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[10] Michaelis: Ztschr. f. Immunitätsforsch., 1912, O., 14, 170.

The effect of hydrogen on bacteria was likewise studied. Hydrogen being only slightly soluble, it was not expected that its effect would be so marked as that of $CO_2$, its solubility being ~~0.031~~ 0.01519 volumes per atmosphere. Bacteria were placed in the apparatus described in the previous experiment and attached to a hydrogen tank under pressure of 120 atmospheres. It will thus be seen that only 2.2 volumes of hydrogen could be dissolved as compared with 52 volumes of $CO_2$. Sterile cultures were never obtained under the influence of hydrogen, although by plating the bacteria before and after treatment it was possible to demonstrate that from 10-40% of B. coli had been killed in 24 hours. There was evidence that many of the bacteria were broken up under the influence of hydrogen and this seemed in particular to be true of the tubercle bacilli. The most marked effect of hydrogen on bacteria, however, was its tendency to affect their staining reaction. Gram-positive organisms would often become gram-negative, and even the acid-fast character of the tubercle bacilli was impaired.

A study was made of the effect of hydrogen on the pathogenicity of pneumococci; it was found, however, that the pathogencity of these organisms for white mice remained unaltered.

Nitrogen under a pressure of 120 atmospheres seemed to be entirely inert. The bacteria were not killed; neither did they present any morphologic change.

## IMMUNIZATION EXPERIMENTS

As stated at the beginning, our object was to obtain the antigenic principle of bacteria in a diffused state in order to make it less accessible to the phagocytes, thereby hoping to obtain an antigen capable of producing a higher degree of immunity than is ordinarily possible with bacterial antigens. Typhoid bacilli which had been under orthostatic pressure of from 6,000-12,000 atmospheres were placed on a shaking machine for 10 hours, after which they were filtered through a Berkefeld filter and the filtrate injected into rabbits.

The question of a standard dosage at once became a serious problem. Bacteria which had been under pressure were difficult to standardize. The following technic was therefore adopted. Six 24-hour slant agar cultures were mixed with infusorial earth and placed under pressure for 14 hours. On removal from the pressure apparatus the infusorial-earth-bacterial mixture was taken up in 50 c c of distilled water and placed on the shaking machine and later filtered. Two c c of the filtrate were then injected into the marginal ear vein of rabbits at 5-day intervals. Ten days following the third injection the agglutinating titer of the blood serum was determined. It was not uncommon to find that the serum of rabbits thus treated would agglutinate typhoid bacilli in dilutions of 1 : 10,000. We rarely found the titer to be below 1 : 5,000. Rabbits treated with living typhoid bacilli in the same manner never yielded a serum of this agglutinating titer: from 1 : 100 to 1 : 500 being the average agglutinating titer of the serum of rabbits treated in this way.

Animals were likewise immunized with bacteria and bacterial filtrate of cultures killed by $CO_2$. Although these experiments are not yet terminated it may be said that bacteria killed by $CO_2$ give excellent results in the way of stimulating antibody formation, it being found that typhoid bacilli killed in this way gave an agglutinating serum of a higher titer than when a corresponding amount of the living culture was used. The B. coli as well as other gram-negative bacilli, it was observed, became far more toxic after having been killed by $CO_2$ than the living cultures. This is probably due to the fact that large amounts of bacterial protein were liberated by the killing process.

A few attempts to immunize with the hydrogen-treated bacteria have been undertaken. In studying the complement-fixation reaction in tuberculosis excellent results were obtained with antigens which had been disintegrated by hydrogen.

## RÉSUMÉ

It has been found that a direct pressure of 6,000 atmospheres kills non-spore-forming bacteria in 14 hours. A pressure of about 12,000 atmospheres for the same length of time is required to kill spores.

Non-spore-bearing bacteria are killed by $CO_2$ of 50 atmospheres pressure in about 1½ hours. Yeast cells withstand the action of $CO_2$ for more than 24 hours, probably because of their ability to transmit the $CO_2$ molecule through the membrane promptly.

The maximum acidity of solutions of $CO_2$ used in our experiments was $P_H$ 10-3.15. It was shown that this degree of acidity was not a factor in killing the bacteria. A solution of sodium chlorid of a molecular concentration corresponding to a solution of $CO_2$ under 67 atmospheres and acidified to $P_H$ 10-3.15 required 15 hours to render cultures of staphylococci and B. coli sterile. It was therefore concluded that the sudden change in the osmotic tension of the fluid in which the bacteria were suspended was the factor which destroyed the organisms.

Many of the gram-negative bacteria could be broken up by the sudden release of the $CO_2$ while the gram-positive bacteria, although killed, suffered little or no morphologic change.

Hydrogen killed from 10-40% of B. coli. This gas had no effect on the pathogenicity of pneumococci.

The acid-fast character of the B. tuberculosis was impaired by the action of hydrogen. Hydrogen-treated tubercle bacilli yielded a suitable antigen for the complement fixation reaction.

Nitrogen under a pressure of 120 atmospheres had no effect on bacteria.

Filtrate of typhoid bacteria which had been subjected to a direct load of 6,000 atmospheres for 14 hours was found to be far superior to the living culture as an immunizing antigen. Bacteria killed by $CO_2$ were likewise found to be excellent antigens.

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# THE PERFUSION EXPERIMENT IN THE STUDY OF CELLULAR ANAPHYLAXIS *

WITH ONE PLATE

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Although a large amount of work has been done in recent years in attempts to determine the fundamental nature of anaphylaxis, this problem has not been entirely cleared up. It is not the purpose of this paper to attempt in any way to review the vast literature which has accumulated on the subject. In the interest of context, however, it may be recalled that there are two theories regarding the fundamental mechanism of anaphylaxis. Friedberger and his pupils hold to the humoral theory. According to this conception, when an antigen is acted on by its specific antibody of the third order the antigen is broken up into toxic substances which are called anaphylatoxin or apotoxin, to which all manifestations of protein anaphylaxis are ascribed. This conception of the mechanism of anaphylaxis would seem to be supported by the test tube experiment, in which apotoxin may be readily formed by bringing together the suitable reagents. The humoral theory is, however, wholly inadequate to account for the observation that, if an animal is immunized passively, a certain incubation period must elapse before anaphylactic phenomena manifest themselves on the introduction of the antigen.

It was a study of the mechanism of passive anaphylaxis that led to the development of the cellular theory, the chief proponents of which are Schultz,[1] Dale,[2] Weil,[3] and Cocoa.[4] According to the cellular theory the site of reaction in anaphylactic phenomena is not in the tissue fluids but within the cells. When an animal is immunized passively it is assumed that the tissue cells appropriate the antibodies and thus become sensitized to the antigen. According to the view of the proponents of the cellular theory of anaphylaxis, we have here

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[1] Jour. Pharm. and Exper. Ther., 1910, 1, p. 549; Hygienic Lab., Bull. No. 80, 1912.

[2] Jour. Pharm. and Exper. Ther., 1913, 4, p. 167.

[3] Jour. Med. Research, 1913, 22, p. 497; 1914, 25, p. 87 and p. 299.

[4] Ztschr. f. Immunitätsforsch. u. exper. Therap., O., 1914, 20, p. 622.

a plausible explanation of the period of latency required to effect passive sensitization, which the Friedberger theory does not supply. If we accept this explanation of anaphylaxis it is apparent that the tissue cells discriminate between the different orders of antibodies, as passive immunization does not always sensitize against the specific antigen. Were this the case it would be the height of folly to administer tetanus antitoxin prophylactically, as such treatment would sensitize the individual to tetanus rather than afford protection. It is, on the contrary, admittedly good therapeutics to immunize passively against tetanus and in some cases against diphtheria as well. The individual immunized against tetanus never becomes more sensitive, but invariably more immune to tetanus toxin. If the tissue cells should appropriate the injected tetanus antitoxin and the individual later become infected with tetanus, the passive immunization would probably prove fatal. It would therefore appear that antibodies of the first order are not involved in the mechanism of cellular anaphylaxis. The question as to the reason for this discrimination by the tissue cells between the antibodies of the first and third order would indeed be an interesting problem for investigation.

While there are some very potent objections to the unreserved acceptance of the cellular theory of anaphylaxis, it has, on the other hand, much in its favor. The fact that a period of incubation of at least from 4-6 hours is required to sensitize passively would seem to allow of no other explanation. This is further supported by the observation of Weil that tissue sensitization is coincident with the disappearance of antibodies from the blood stream. Weil found further that a sensitized animal could be protected from anaphylaxis by insuring the presence of antibodies in the blood stream when the antigen was given.

The final supporting pillar to the theory of cellular anaphylaxis was furnished by the work of Schultz and Dale who attempted to wash the sensitized tissues free of blood and lymph, after which such tissues were exposed to the stimulus of their specific antigens. These experiments presuppose the ability completely to isolate the tissue cells from the body fluids. Dale washed the uterus of the guinea-pig supposedly free of blood by inserting a cannula into the abdominal aorta and perfusing the lower half of the body with Locke's fluid, allowing the efferent fluid to escape from the inferior vena cava. The perfusion was continued "for upwards of half an hour" until the efferent fluid appeared free of blood. The same technic has been used by Weil and others in the study of cellular anaphylaxis in guinea-pigs.

No attempt was made by these authors to estimate the amount of residual blood remaining in the tissues following perfusion. Such investigation was undertaken by Cocoa. By estimating the amount of blood cells and complement in the perfusion fluid he arrived at the conclusion that from 1.6-6% of residual blood remained in the tissues following perfusion.

Manwaring[5] perfused the lungs of normal and anaphylactic guinea-pigs by putting the afferent cannula into the pulmonary artery and allowing the fluid to escape through an incision in the left auricle. Assuming that he had washed the lungs free of blood with Locke's solution, he concluded that the anaphylactic reaction was partly cellular and partly humoral.

In studying spontaneous phagocytosis in the liver with India ink we were surprised to find that the ink particles were deposited only in restricted areas of the organ. It was evident that only a small part of the liver was traversed by the perfusing fluid. This observation suggested the possibility of making use of this procedure in studying the course of the perfusing fluid through an organ and in determining to what extent it is possible to wash an organ free of blood.

## EXPERIMENTS

The apparatus is so constructed that fluid, at body temperature, may be passed through the organ and collected for examination. The animal is placed inside an incubator kept at a temperature of 37 C. A funnel provided with a stopcock is supported on a ring-stand. Fluid from the funnel passes through a glass coil which rests in a beaker of water kept at a temperature of 38 C. A rubber tube leads from the glass coil through a small hole in the side of the incubator to the afferent cannula. This tube is connected with a mercury manometer and the height of the funnel is so adjusted that the manometer registers a pressure of about 2-3 cm. Hg. The efferent cannula is connected with a rubber tube which passes through a small hole in the side of the incubator so that the fluid may be collected outside. Several places are provided to let out any air bubbles which may get into the apparatus. By means of the stopcock on the funnel the perfusion may be discontinued temporarily at any time. Locke's solution, at a temperature of about 37 C., was used to perfuse the organs. Most of the experiments were performed on the liver, but the lungs and the uterus were also perfused a few times each. A typical experiment will illustrate the procedure and the results.

The rabbit is killed with ether. Before the heart quits beating a cannula containing sodium citrate is inserted into the portal vein near its entrance into the transverse fissure of the liver. The gastric vessels in the lesser omentum are then tied off. Another cannula is inserted into the superior vena cava in the thorax. From 50-100 c c of Locke's solution warmed to 37 C. are passed through immediately under gentle pressure to wash out most of the blood. The animal is then put into an incubator (temperature 37-40 C.).

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[5] Jour. Immunol., 1917, 2, p. 157.

and the perfusion is begun at once, about 10 minutes after the insertion of the first cannula. The manometer registers a pressure of about 2-3 cm. Hg. Perfusion begins at 2:55. Fluid from exit cannula is reddish at first but finally becomes clear. At 3:07 a faint trace of albumin in fluid from exit cannula; 3:15, albumin negative. Perfusion stopped for 3 minutes, then continued. Fluid is now blood stained, shows erythrocytes microscopically, and gives positive albumin test. 3:22, albumin negative; perfusion stopped for 5 minutes. 3:27, perfusion begun again. Fluid contains blood. Albumin strongly positive. 3:35, albumin negative. Perfusion discontinued for 6 minutes. 3:41, perfusion continued. Blood and albumin present. 3:48, albumin negative. Perfusion discontinued for 5 minutes. At 3:53, perfusion continued. Blood and albumin present. 4:00, albumin negative. Perfusion discontinued for 5 minutes. 4:05, perfusion continued. Blood and albumin present. 4:06, end of experiment. About 2,000 c c of Locke's solution has been passed through the liver.

All perfusions, whether of liver, lung, or uterus, give about the same results. It is never possible to wash out all the blood and albumin. The fluid from the exit cannula soon becomes free of albumin and blood, but if the perfusion is discontinued for a few minutes both these substances reappear. The explanation of this phenomenon will be more apparent after we have considered the experiments with India ink.

### Typical India Ink Experiment

A liver is prepared as described above and washed with 1,000 c c of Locke's solution. The fluid from the exit cannula is free from albumin. Then a litle India ink (Higgins) is added to another 1,000 c c of Locke's solution and this is passed through the liver. The ink suspension is very thin. At first the fluid from the exit cannula is clear, all the ink particles having been taken up by the endothelial cells of the liver. Later on the fluid from the exit cannula is just as dark as that entering the afferent cannula, showing that no more ink particles are being held in the liver. The perfusion is discontinued at this point. The pressure is constant throughout the experiment and the rate of flow from the exit cannula is unchanged, indicating that there is no obstruction to the flow of the fluid.

A liver perfused with Locke's solution containing India ink shows blackened portions, the black color being due to retention of the carbon particles of the ink by the endothelial cells of the liver. The blackened areas evidently correspond to the parts of the liver through which the perfusing fluid passed. There is great variation in the size and position of the blackened areas in different specimens. In every case there are large areas of liver tissue not blackened at all. In some instances only the dorsal part of the liver is blackened (this is the lower part during the perfusion); in others only a comparatively small area around the hilus. The blackened areas shown in Figs. 1, 2, 3, and 4 indicate the parts of the liver through which the perfusing fluid actually passed. It is clear that even in the most favorable cases there are extensive areas of liver tissue not reached by the perfusing fluid and that sometimes only an insignificantly small part of the liver is perfused. Even in blackened portions of the liver the cut surface shows that the fluid has reached only the central parts of the lobule (Fig. 5). It is therefore an error to assume that all the blood has been washed out of the liver because the fluid coming from the organ is free from albumin.

It was pointed out in the foregoing that it is not possible to wash all the blood out of the liver. It is easy to get the fluid from the liver free from albumin by continuous rather rapid perfusion; but if the perfusion is discontinued for a few minutes blood and albumin invariably reappear. The experiments with ink give us a clear explanation of this phenomenon. The perfusing fluid traverses only certain parts of the liver and these soon become free of blood; but during the periods when the perfusion is discontinued, blood from the adjacent areas of liver tissue diffuses into the main path of the fluid. Hence, at each renewal of the perfusion after a pause blood and albumin reappear.

On theoretical grounds it is not to be expected that all of the blood can be washed out of an organ with Locke's solution since the latter is much thinner than blood and produces much less friction on the walls of the vessels. As soon as a passage is opened up through the organ the resistance along this route will be much less than through capillaries containing blood, and the fluid will take the path of least resistance.

Air bubbles cannot be a factor in directing the course of the perfusing fluid since these were always carefully excluded. The ink does not block any of the blood vessels. The carbon particles are found on microscopic examination for the most part in the endothelial cells. A liver blackened by an ink perfusion is unaffected by washing with clear Locke's solution. No carbon particles are washed out.

The lungs were perfused by putting the afferent cannula in the pulmonary artery and the efferent in the left auricle. The trachea was ligatured before the thorax was opened to prevent collapse of the lungs. It was also found impossible to wash all the blood out of the lungs. The efferent fluid soon became free of blood and albumin, but both these substances reappeared after temporary interruption of the perfusion. Perfusion with India ink, after preliminary washing with Locke's solution, showed the same irregular blackening of the lung tissue as was described above in the liver (Fig. 6).

The uterus was perfused according to Dale's method. In general, the same results were obtained as with the lungs and the liver.

Since the perfusion of an organ is so incomplete it is obvious that circulating antibodies are not entirely removed by this procedure. It follows that the experiments of Dale and Weil with perfused uterine muscle cannot be regarded as lending any support to the theory of cellular anaphylaxis. In like manner it will be seen that the presence of cellular antibody is not established in Manwaring's experiment with 14-day anaphylactic lung.

It is recognized by us that our experiments do not overthrow the doctrine of cellular anaphylaxis. It is our purpose only to call attention to the unsoundness of one of the main arguments which have been advanced in its support.

## SUMMARY

It has been shown that it is impossible to wash all the blood out of an organ by perfusion methods. The efferent fluid becomes free from blood and albumin in a short time, but these substances reappear if the perfusion is temporarily discontinued a few minutes.

By perfusing organs with Locke's solution containing India ink, the course taken by the fluid through the organ has been mapped out. It has been found that a surprisingly small part of the capillary system is really washed out by the perfusing fluid.

This technic does not therefore remove circulating antibodies completely, as has been assumed, and this type of experiment does not establish the presence of cellular antibodies.

## EXPLANATION OF PLATE

The organs were first perfused continuously with warm Locke's solution until the efferent fluid was free from albumin, after which they were perfused with warm Locke's solution to which some India ink had been added. The blackened areas show the course of the fluid through the organ. All figures are photographs.

Fig. 1. Ventral surface of liver.

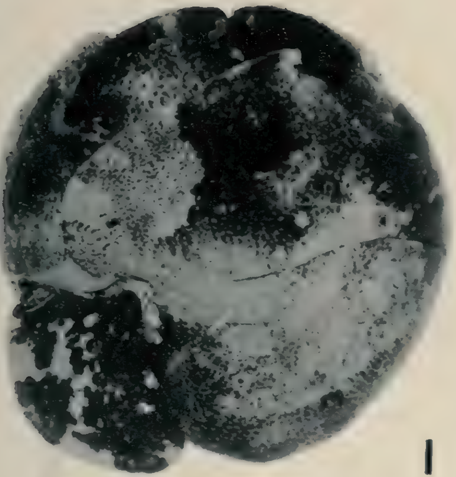
Fig. 2. Dorsal surface of liver. Very little passed through the left lobe.

Fig. 3. Two pieces of a liver, showing a very incomplete perfusion.

Fig. 4. Dorsal surface of liver. The fluid passed through a relatively small area around the transverse fissure.

Fig. 5. Section of a deeply blackened area from a liver, showing that even in this part not over half the hepatic cells are reached by the perfusing fluid.

Fig. 6.—Dorsal surface of lungs. Very incomplete perfusion of one lung.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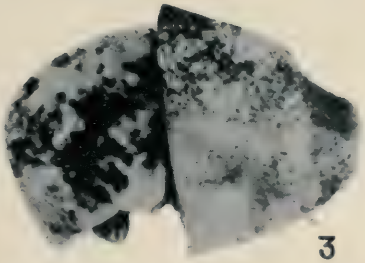
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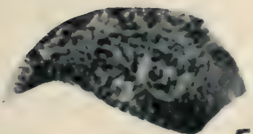
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# The Influence of the Surface Tension of the Culture Medium on the Growth of Bacteria

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AND

T. B. HARTZELL

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# THE INFLUENCE OF THE SURFACE TENSION OF THE CULTURE MEDIUM ON THE GROWTH OF BACTERIA *

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There is probably no phase of bacteriologic research that has occupied the attention of investigators more than the preparation of culture mediums. Most of such investigations have been directed toward supplying bacteria with the nutritive material best adapted to their specific individual requirements. Aside from its reaction, the physico-chemical state of the medium has received but little attention.

It is the purpose of this paper to record the behavior of some of the common bacteria when cultivated on medium of low surface tension.

Our investigation naturally began with a search for suitable substances with which to lower the surface tension of mediums. It was soon found that substances that would lower the surface tension of water would not always act satisfactorily in lowering the tension of broth or other mediums. Furthermore, only such surface tension depressants could be used as were of themselves toxic neither to bacteria nor to laboratory animals into which cultures or filtrates were subsequently to be injected. It was found that soap fulfilled our requirements better than anything else tried. A large variety of commercial soaps were used, after first being purified and neutralized. They all possessed certain disadvantages, however, as they invariably caused a marked turbidity of the medium. Furthermore, the surface tension of the medium depressed by the various soaps would not remain constant, due to the fact that soap tends to hydrolyze when diluted, which causes the tension to rise. Studies were therefore made in an effort to secure a soap that would be perfectly clear when in solution, and which would be sufficiently stable to prevent the objectionable fluctuations in the surface tension of the medium. It was found that pure castor oil saponified with $NaOH$ met these requirements.

A number of neutralized fatty acids form a clear solution when hot, but become turbid and viscous on cooling. Solutions of castor oil soap

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on the other hand remain perfectly clear when cooled, do not hydrolyze on sterilization in flowing steam, nor do they become viscous when cooled.

Castor oil soap is prepared by adding an alcoholic solution of NaOH to castor oil slightly in excess of its saponification equivalent. The mixture is then heated over the water-bath until saponification is complete when the soap is dissolved in hot absolute alcohol to which is added a few drops of phenolphthalein. Hydrochloric acid, diluted with alcohol, is then added drop by drop until the soap solution is neutral. It is then filtered on paper to remove the chlorids. The excess of alcohol may then be removed by evaporation, after which the soap solution is poured into a saturated solution of NaCl to remove the glycerol and other impurities. After further filtration followed by washing with a saturated solution of sodium chlorid the soap is ready for use.

The drop method was used in measuring the surface tension.

$$\frac{\text{Weight of number drops of medium}}{\text{Weight of number drops of water}} \times 73$$

In order to give a general idea of the comparative surface tension of various fluids it may be stated that that of water is 73, alcohol 22, and ether 16 dynes.

The broth prepared in our laboratory by standard methods has a surface tension of about 59 dynes. This may vary slightly, but it was always found to be between 58 and 60 dynes, 59 being the value usually obtained. The surface tension of broth may be lowered to any desired point down to 32 dynes by adding the necessary quantity of soap solution to the medium.

In growing bacteria on broth of lower surface tension we were impressed by the behavior of the B. subtilis when the tension had been depressed below 45 dynes. When grown on such medium this organism no longer forms a pellicle, but grows down in the body of the medium. When the tension of the broth is depressed to below 45 dynes the growth of the B. subtilis is largely at the bottom of the tube. It was further observed that spore formation was markedly suppressed and sometimes entirely absent, especially after cultivation on low tension mediums for several generations. B. subtilis grown on medium of low surface tension may sometimes be rendered sterile by heating to 60 degrees for an hour which indicates that spore formation has been entirely suppressed. It is generally supposed that B. subtilis grows on the surface of broth because of its great demand for oxygen.

Since our work has shown that this organism is capable of growing throughout the medium, even at the bottom of the tube, it is evident that its oxygen requirement is not the chief reason for surface growth on ordinary broth. In this connection we would not overlook the fact that lowering the surface tension of water increases its ability to dissolve oxygen and other gases. A striking feature common to most bacteria which grow on the surface of liquid mediums, particularly B. subtilis, B. mesentericus, and B. tuberculosis and others, is that whenever there is pellicle formation there is little or no growth except at the very surface. In inoculating a flask of glycerol broth with B. tuberculosis large numbers of organisms may sink to the bottom of the flask, but only those that remain at the surface show signs of multiplying. Other bacteria, such as B. coli, the cholera spirillum, first show signs of development at or near the surface even though there is no pellicle formation. What can be the explanation of this selective site of so many bacteria? It is possible that at the surface of the medium certain substances are concentrated which favor, or are even necessary, for the metabolism of many micro-organisms. It is a well known fact that surface energy tends to reduce itself to a minimum, and that this may be accomplished in two ways: (1) by the reduction of the surface area of the liquid, or (2) by reducing the surface tension, or both. The surface tension of the liquid is reduced to the lowest point possible through the concentration of the surface tension depressants at the surface of the liquid. B. subtilis and other bacteria, which show such predilection for the surface of the medium, probably select this zone because the surface tension reducing substances concentrated there are required for their metabolism. The pellicle formation is probably a mere coincident, the surface tension of the fluid being sufficient to support the weight of the organisms just as certain insects may be supported on the surface of water because of its high surface tension.

The question naturally suggests itself as to whether or not all aerobic bacteria might not become pellicle formers were the surface tension of the culture medium sufficiently high. Since it is impossible to raise the tension of a medium much above that of water, this experiment has its limitations. However, we have found that B. coli as well as some of the paratyphoid bacteria form well developed pellicles when grown on an inorganic medium having a surface tension of 72.2 dynes.

Investigations have also been conducted with reference to the influence of the surface tension of the medium on some of the common anaerobic bacteria, such as B. tetani, B. botulinus, B. chauvei. It was found that B. tetani grew well under ordinary aerobic conditions providing the surface tension of the medium had been properly depressed. B. tetani produces a potent toxin when grown on ordinary broth, the surface tension of which has been reduced to about 40 dynes.

This would seem to indicate that we cannot draw a sharp line between the aerobic and anaerobic bacteria although there can be no question but that some bacteria demand a liberal supply of oxygen. while others cannot thrive where the supply is too liberal. In this connection it may be pointed out that one of the popular methods of producing anaerobiosis is to cover the medium with a layer of sterile oil, the object of the oil being to exclude oxygen from the medium which it covers.

Since oil is a much better solvent of oxygen than water, is it reasonable to suppose that oil actually does exclude oxygen? If the tube were sealed off immediately after introducing oil into the test tube it might be argued that the oil, because of its being a better oxygen solvent than the medium, would cause the gas to diffuse from the medium into the oil later; but since the sealing of the tube following the introduction of the oil is not necessary to effect the so-called anaerobic condition, this is obviously not the explanation. A little experiment which shows that the oil does not exclude oxygen from culture medium may be made by inoculating a tube of broth with a culture of B. subtilis after which the inoculated medium may be covered with a layer of sterile oil; 48 hours following this inoculation it will be found that the subtilis has formed a very heavy pellicle just beneath the oil layer, showing that oxygen does pass from the oil into the medium, otherwise this organism would not be able to grow at this point. The oil undoubtedly favors the growth of anaerobic bacteria because it reduces the surface tension of the medium, and as a result the surface tension depressants contained in the medium are not concentrated at the surface as would ordinarily be the case, but are dispersed throughout the medium where they can serve the metabolic requirements of the anaerobic bacteria that under ordinary circumstances would be unable to avail themselves of these surface tension lowering substances because of the excess of oxygen at the surface where they are concentrated.

The mere act of lowering the surface tension of the medium will not cause all anaerobes to grow under ordinary aerobic conditions. Such a germ as B. chauvei fails to show any appreciable development under such conditions.

Most bacteria seem to grow best when the surface tension has not been depressed below 53 to 55 dynes, although most of the more common bacteria, such as the staphylococcus, B. coli, B. typhosus, and B. paratyphosus show some growth when the surface tension has been depressed as low as 32 dynes.

Surface tension below 45 dynes tends to cause many bacteria to agglutinate, thus giving the impression that the growth is entirely at the bottom of the test tube, this being particularly true of the typhoid and paratyphoid bacilli. The streptococci and pneumococci do not grow well where the surface tension has been brought below 50 dynes. The pneumococci show a tendency to disintegrate when grown on a medium of a low surface tension. This observation suggests that the solution of pneumococci by bile may be due, in part at least, to the lowering of the surface tension which bile induces. B. tuberculosis grows very well on medium of low surface tension. Like B. subtilis it no longer grows with pellicle formation, but grows at the bottom of the medium.

The pathogenicity of bacteria seems to be profoundly affected by the surface tension of the medium on which they are grown. We hope to give a detailed report on the relation of pathogenicity and toxin formation of bacteria to the surface tension of the medium in a subsequent article.

## SUMMARY

The growth of bacteria on ordinary broth is greatly influenced by the surface tension of the medium.

A sodium soap of castor oil proved to be a practical surface tension depressant.

All pellicle formers cease to grow at the surface where the tension is below 45 dynes.

The tendency of B. subtilis to form spores is considerably reduced when the organism is grown on broth medium of low surface tension.

Some anaerobes, B. tetani in particular, grow well under ordinary aerobic conditions on medium of reduced surface tension. The layer of oil often used to cover mediums for the purpose of producing anaerobiosis, probably acts by reducing the surface tension rather than by excluding oxygen.

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# MULTIPLE VASCULAR ANOMALIES OF THE GREAT VESSELS IN A NEWBORN CHILD.*

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(With four illustrations.)

## I. INTRODUCTION.

In recent years studies on the vascular system of higher vertebrates have gone far in establishing the methods by which vessels grow, as well as the modes of derivation of the definitive trunks from the primitive vessels. These studies, which have been carried on by a large number of investigators by the application of various methods, have led us insistently to the conclusion that the vascular system of the individual is fashioned during embryonic life according to a definite plan and pattern which strikingly manifests the effects both of phylogeny and heredity. The old hypothesis introduced by Abey (1868), "of a uniform all-pervading capillary plexus *anlage* for the vascular system, is far too crude and inexact for the facts" Evans (1912). Some of the most convincing evidence against this old theory is that obtained from the study of the anomalies which the vascular system presents.

In reporting the present case the authors have not attempted to review the literature extensively, neither have we purposed to discuss at length the possible interpretations of the anomalies. Believing that the individual case carefully investigated and recorded in the literature has its intrinsic value, we have been content to describe and offer a simple interpretation of the conditions found. When many more such cases have been given literary permanence, it will remain for some later worker to assemble them in a comprehensive synthetic study from the magnitude of which far more accurate and significant conclusions may be drawn than by the study of a single case.

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* From the Department of Pathology, University of Minnesota.

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The specimen about to be described is the body of a female infant born alive at term, but which lived only a few minutes after delivery. The body measured 44 centimeters, total length, and weighed 3000 grams. It was sent into the Department of Pathology of the University of Minnesota for postmortem examination. Besides the vascular anomalies which are fully described below, malformations affecting other systems were also present. These were as follows: slight macroglossia, imperforate anus, bicornate uterus, and hypoplasia of the right kidney. Despite the unusual vascularization of the lungs and of the left lower extremities these structures were normally developed.

## II. DESCRIPTION OF ANOMALIES.

I.—Arterial anomalies. *a.* Aplasia of the pulmonary arteries. The conus arteriosus arises from the right ventricle, as shown in Fig. 1, and passes cranially and to the left to be continued as the ductus arteriosus. The ductus joins the descending portion of the aortic arch at the point marked 13 in Fig. 1, forming with the latter vessel a very acute angle. In this specimen the conus and ductus arteriosus constitute, in reality, one trunk whose caliber is uniform throughout its entire course. No branches are given off at any point and it is therefore impossible to say at what place the conus terminates or the ductus takes origin. No trace of the right or left pulmonary artery can be found either by external examination of the trunk or by a study of its intimal surface.

*b.* Dysplasia of the left sciatic and femoral arteries with aplasia of the left common iliac, external iliac, and umbilical arteries.

As shown in Fig. 2, the abdominal aorta (A), in the upper part of its course, gives origin to its usual branches in their accustomed order and position. Just caudal to the point of origin of the superior mesenteric artery (Fig. 2, 2) the aorta is slightly deflected toward the right. From this region it passes obliquely across the anterior surface of the vertebral column, giving rise in its course to the inferior mesenteric artery (Fig. 2, 3) and terminating at the level of the lower margin of the second lumbar vertebra by dividing into two very unequal trunks—the right common iliac artery (Fig. 2, 5) and the left sciatic artery (Fig. 2, 4). Of these terminal trunks the right and larger vessel, which appears as the direct continuation of the aorta, is regarded as the right common iliac. It passes downward and laterally, maintaining the general course of the parent trunk, and bifurcates in front of the iliosacral joint at the level of the inferior edge of the fourth lumbar vertebra into the right umbilical (Fig. 2, R. U. A.) and the right external iliac arteries (Fig. 2, 6). The right umbilical artery, which is the larger of these two branches, passes into the pelvis where it gives off four small vessels. After passing along the medial pole of the right ovary and under the right horn of the uterus just medial to the uterine attachment of the round ligament, it is continued onto the anterior abdominal wall. Here the vessel runs obliquely upward and somewhat medially to reach the umbilicus through which it courses. The right external iliac artery is the smaller of the terminal branches

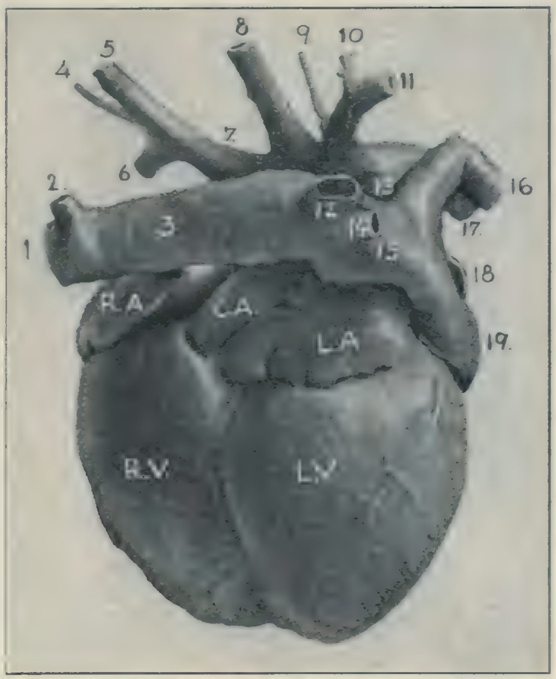

FIG. 1.—Antero-left lateral view of the heart and great vessels. R. A., right atrium; L. A., left atrium; R. V., right ventricle; L. V., left ventricle; C. A., conus arteriosus; 1, right subclavian vein; 2, right internal jugular vein; 3, right innominate vein; 4, right vertebral artery; 5, right common carotid artery; 6, right subclavian artery; 7, innominate artery; 8, left common carotid artery; 9, left vertebral artery; 10, left thyreocervical trunk; 11, left subclavian artery; 12, left internal jugular vein; 13, ductus arteriosus; 14, left subclavian vein; 15, left superior vena cava; 16, hemiazygos vein; 17, descending portion of aortic arch; 18, lower left pulmonary vein; 19, coronary sinus. × 1.4.

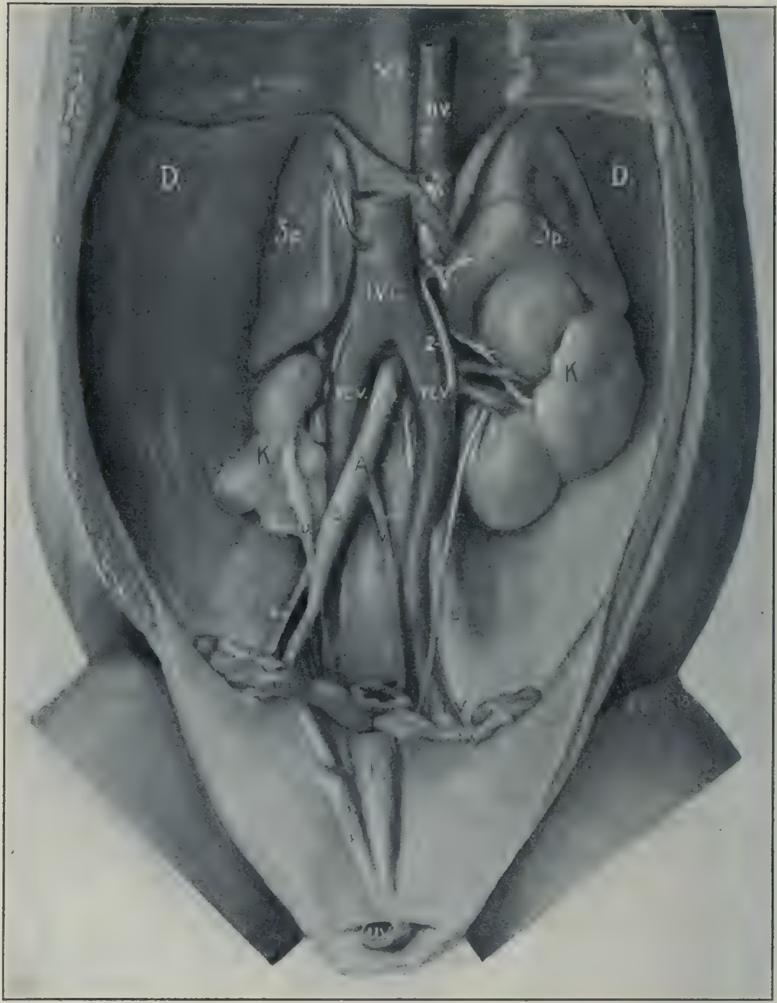

FIG. 2.—Drawing of a dissection of the structures in the posterior part of the abdominal cavity and the lower thoracic cavity; dissected to show the vascular elements of these regions and their relations. A., aorta; B., bladder; D., diaphragm; E. I. V., external iliac vein; H. V., hemiazygos vein; I. V. C., inferior vena cava; K., kidney; O., ovary; P. C. V., posterior cardinal vein; R. U. A., right umbilical artery; Sp., suprarenal gland; U., ureter; U. T., uterine tube; V. C., vertebral column; 1, celiac axis; 2, superior mesenteric artery; 3, inferior mesenteric artery; 4, left sciatic artery; 5, right common iliac artery; 6, right external iliac artery. × 1.

of the common iliac. It passes forward and laterally along the superior aperture of the minor pelvis, resting in the groove between the external iliac vein medially and the psoas muscle on its lateral side. After its course under the ovary and the uterine tube it passes beneath the inguinal ligament to become the femoral artery.

The left terminal branch of the aorta, as pointed out above, is considered to be the left sciatic artery and presents quite different relations from those of the right terminal division. As shown in Fig. 2, the size of this vessel is much less than that of the right common iliac. It passes from the point of origin directly into the lower part of the true pelvis. In its course, besides giving origin to a single minute branch which arises just after the sciatic leaves the aorta and which passes for a short distance along the anterior surface of the third lumbar vertebra as the midsacral artery, three left lumbar arteries are given off, as well. It runs along the posteromedial aspect of the left posterior cardinal vein, enters the pelvis medial to the left hypogastric vein, and passes to the region of the left sciatic foramen. In the region of the sciatic foramen a branch, given off to become the inferior gluteal artery, accompanies the sciatic nerve through the foramen. The inferior gluteal artery is a very small vessel—the major part of the sciatic trunk having an entirely intra-abdominal course. From the point at which the inferior gluteal arises, the larger vessel turns forward and, passing between the sciatic nerve on its medial side and the obturator nerve on its lateral side, it turns upward along the anterior part of the left lateral wall of the pelvis to associate itself with the terminal portion of the left external iliac vein. It then turns sharply downward to be continued into the thigh as the femoral artery. The angle formed at the point of origin of the femoral artery is one of approximately 90 degrees. In the thigh the left femoral trunk assumes its ordinary relations, being enclosed in the medial compartment of the femoral sheath.

The distal portions of the left common iliac, the left external iliac, and the left umbilical arteries are completely absent.

2. Venous anomalies. *a*. Persistence of the left cardinal system with aplasia of the right superior vena cava.

The great venous trunks in the region of the heart present numerous anomalies. The right superior vena cava is entirely absent. The right internal jugular and the right subclavian veins unite at the sternal end of the clavicle to form the right innominate vein. This trunk is much longer than in a normal specimen and presents quite different relations. It courses almost horizontally across the anterior aspect of the base of the heart, passing in front of the right atrium, the conus arteriosus, and the ascending portion of the aortic arch, and receives the right and left inferior thyroid veins as tributary branches. In the region posterior to the left sternoclavicular junction and in front of the medial margin of the left atrium the trunk is joined by a very short vessel (left innominate vein) formed By the union of the left internal jugular and left subclavian veins. by the confluence of the two innominate trunks the left superior vena cava is formed and is immediately joined on its posterior aspect by the large hemiazygos vein. The vessel thus formed passes obliquely downward lateral to the left side of the left atrium and in front of the lower left pulmonary vein. It reaches the coronary sulcus through which it runs as the coronary sinus and terminates by opening into the right atrium just to the left of the inferior vena cava orifice. The hemiazygos vein, which begins in the region of the left kidney, is a relatively large trunk (Fig. 2). It forms anastomoses with the upper lumbar veins, pierces the left crus of the diaphragm and, in the thoracic cavity, is joined by the much smaller azygos vein from the right side, as well as by the left bronchial veins and a number of left intercostal veins. Its termination in the left superior vena cava, which was described above, is shown in Fig. 1.

The inferior vena cava, which is present in this specimen as a relatively short trunk, is formed by the union of the two posterior cardinal veins. The trunk thus formed courses cranialwards, resting upon the anterior surface of the vertebral column, and pierces the diaphragm approximately in the midline. Its intrathoracic portion is very short and the vessel terminates by opening into the lower extremity of the right atrium. This trunk receives as its tributaries not only the posterior cardinal veins but also the right suprarenal and the hepatic veins.

The posterior cardinal veins are bilaterally symmetrical but their relations, as determined by the asymmetry of contiguous structures, are quite different on the two sides. The right posterior cardinal in the upper part of its extent lies immediately to the right of the aorta, while in its lower portion the latter vessel crosses the cardinal and comes to lie anterior to it. The left posterior cardinal vein, on the other hand, is not immediately related to the aorta in any part of its course except at the point at which it joins its fellow of the opposite side. It passes caudalwards along the posterior abdominal wall and at the level of the fourth lumbar vertebra associates itself with the sciatic artery, lying lateral and slightly anterior to that vessel. The two posterior cardinal trunks arise in the pelvic region by the union of the hypogastric and external iliac veins of their respective sides. They also receive as tributaries a number of small vessels from the posterior abdominal wall and the efferent branches from the kidneys.

## III. EMBRYOLOGICAL INTERPRETATION OF THE ANOMALIES.

A rational interpretation of the vascular anomalies described above can only be adduced when their ontogeny is perfectly understood, and our ability to interpret them seems to vary directly with the completeness of our knowledge of the normal developmental processes.

The aplasia of the pulmonary arteries results from some interference with the normal course of development of the sixth pair of aortic arches. On the right side either the sixth arch totally failed of development or this structure may have been lost at an early stage. On the left side, however, the presence of the ductus arteriosus, joining the conus arteriosus and the aorta, indicates that the sixth arch on this side must have been formed, and the absence of the left pulmonary artery seems to be explained only be assuming that the definitive branch to the left lung failed to develop.

The complete aplasia of the pulmonary arteries is apparently an unusual anomaly. For a study of the abnormalities arising in the development of the aortic arches and the literature considering such conditions the reader is cited to the work of Poynter (1916).

The specimen described above presents an instance in which the left sciatic artery arises directly from the aorta. This condition, which has been described by Hochstetter (1890) in mammalian embryos, apparently has never been observed in man. Our knowledge regarding the ontogeny of the posterior limb vessels in man is as yet very imperfect and any explanation of anomalous formations must be based largely on the few more complete studies of comparative forms. The accompanying diagram (Fig. 3) offers our interpretation as regards the development of this unusual type of vascular system to the left lower extremity. The solid lines in the diagram express the conditions as they exist in the specimen and the broken lines indicate the condition of normal development as it is supposed to occur. It would seem that the distal portion of the common iliac, the external iliac, and the umbilical arteries had dropped out at a certain stage of development and that, in consequence of the obliteration of these paths, the primitive sciatic trunk had been retained. But only the intra-abdominal part of this trunk has persisted as a major vessel, for, as shown in the diagram and described above, a secondary connection, by way of a junctional vessel, has been established between the sciatic and the femoral arteries. As a result of this arrangement, the condition which at first seems to be a persistence of the most primitive circulation of the limb is, in reality, only the retention of the proximal portion of the primordial vascular trunk, and the development of the junctional vessel so brings it about that the limb receives its ultimate major vascularization through the femoral quite like the usual case. The inferior gluteal artery, which is the final derivative of the distal portion of the sciatic trunk, exists in this specimen as a relatively minor vessel.

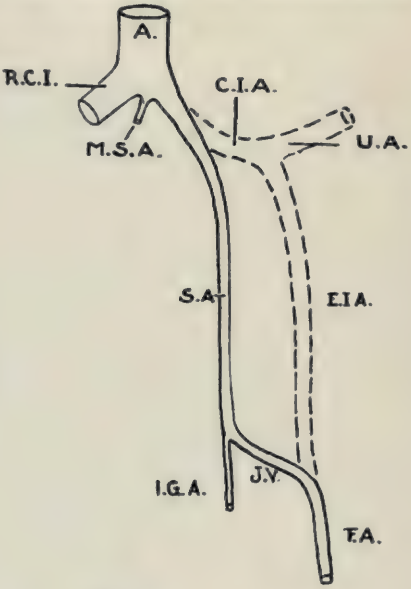

Fig. 3.

Fig. 3.—Diagram of the arterial anomalies of the vascular elements to the left lower extremity. The solid lines express the conditions as they are found in the specimen; the broken lines indicate the parts normally developed but which are absent in this specimen. A., aorta; C. I. A., common iliac artery; E. I. A., external iliac artery; F. A., femoral artery; I. G. A., inferior gluteal artery; J. V., junctional vessel; M. S. A., midsacral artery; R. C. I., right common iliac artery; S. A., sciatic artery; U. A., umbilical artery.

The numerous venous anomalies involving the great vessels around the heart create a condition quite different from the normal.

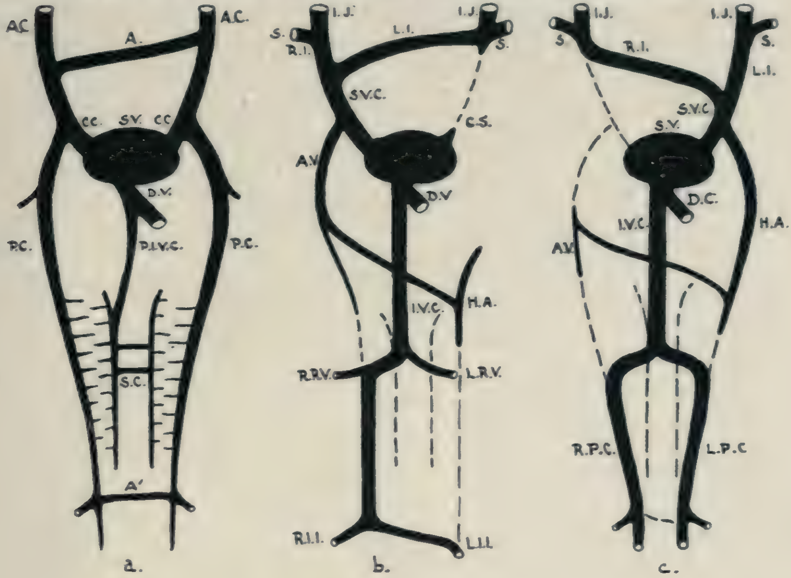

Fig. 4.

FIG. 4.—A series of diagrams expressing the authors' interpretations of the methods of development of the multiple venous anomalies described.

(*a*) Diagram of the venous system of a very young embryo.

(*b*) Diagram of the venous system as it exists in normal ontogeny after the definitive trunks (structures in solid black) have been derived from the primitive system shown in (*a*). The broken lines indicate the parts of the primitive system which are ordinarily lost in the developmental process.

(*c*) Diagram of the venous system as it exists in the specimen. The broken lines indicate the parts of the primitive system shown in (*a*), which have been lost in the developmental process.

A., anastomotic communication between anterior cardinals; A'., anastomotic communication between posterior cardinals; A. C., anterior cardinal; A. V., azygos vein; C. C., common cardinal; C. S., coronary sinus; D. V., ductus venosus; H. A., hemiazygos vein; I. J., internal jugular; I. V. C., inferior vena cava; L. I., left innominate; L. I. I., left internal iliac; L. P. C., left posterior cardinal; L. R. V., left renal vein; P. I. V. C., primitive inferior vena cava; R. I. I., right internal iliac; R. I., right innominate; R. P. C., right posterior cardinal; R. R. V., right renal vein; S., subclavian; S. C., subcardinal veins; S. V. C., superior vena cava.

The anatomical relations which appear to be so widely at variance from the ordinary arrangement can be readily explained only on the basis of the ontogenetic history of the venous system. The accompanying diagrams express our interpretation as regards the mode of development of these conditions. Fig. 4*a* is a schematic representation of the venous system as it exists in young embryos. This primitive system is composed of the bilaterally symmetrical anterior and posterior cardinal veins, which open on either side into the sinus venosus through the medium of the ductus Cuvieri (common cardinal veins). There is also present at this stage an anastomotic communication between the anterior cardinals at points a little cephalad to the heart as well as the transitory subcardinal vessels. It is by means of the alterations occurring in this simply constructed system that the definitive vessels are formed. These well-known changes, which are diagrammatically represented in Fig. 4*b*, need not be recounted here. Fig. 4*c* shows schematically the anomalous arrangement of the vessels found in the case under discussion and its appearance contrasts strikingly with the normal condition shown in Fig. 4*b*. According to our interpretation the anomalous complex must have passed through a stage of development which presented structural arrangements quite like those diagrammed in Fig. 4*a*. In other words, the earliest development of the venous system in this case appears to have been normal and the anomalies seem to date to that period of ontogeny in which the transformations directed toward the derivation of the definitive trunks occur. As is clearly shown by comparison of Figs. 4*b* and 4*c*, that portion of the right anterior cardinal vein which intervenes between the right extremity of the anastomotic branch and the sinus venosus has been lost, thus explaining the absence of the right superior vena cava. The anastomotic channel has persisted on the right innominate vein. The entire left cardinal system has been retained almost without change. The intermediate portion of that part of the left anterior cardinal vein which intervenes between the left extremity of the anastomotic branch and the sinus venosus, which is ordinarily lost, is retained as the left superior vena cava. The left superior vena cava, as pointed out in the above description, opens directly into the heart through the coronary sinus. The cardiac extremity of the left posterior cardinal has persisted so that the hemiazygos vein joins the left superior vena cava just before that trunk opens into the coronary sinus.

McCotter (1916) has assembled the recorded cases of similar venous anomalies and the reader is cited to his paper for a literary review of the subject.

The interpretation of the anomalies related to the ontogeny of the inferior vena cava is shown graphically in Fig. 4. In tracing this trunk caudally from the heart it appears that the cranial portion, which is formed in conjunction with the hepatic circulation of the embryo, has developed in the usual way. That portion of the inferior vena cava derived from the right subcardial and right posterior cardinal veins also shows nothing exceptional. On the left side, however, the caudal part of the posterior cardinal, which ordinarily undergoes complete degeneration, has persisted and is joined to its fellow of the opposite side by the persistent anastomotic channel located between the subcardinal and posterior cardinal systems. The anastomotic vessel between the caudal extremities of the postcardinals, which ordinarily forms the proximal part of the left common iliac veins, either never developed or has been lost without trace.

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# INFLUENZAL SINUS DISEASE AND ITS RELATION TO EPIDEMIC INFLUENZA *

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A typical attack of influenza usually begins as a rhinitis and spreads from the nasal mucosa throughout the respiratory tract. As the chief symptoms are produced by the involvement of the bronchial tree, attention is naturally directed to the lungs and bronchi as the most important foci for the infection. However, in studying acute cases one is often impressed by the fact that the severity of the disease, best evidenced by the general prostration of the patient, is out of all proportion to the physical signs. Tracheobronchitis with abundant mucopurulent sputum, a particularly distressing and often spasmodic cough, and moist râles uniformly present in both lungs, almost exhaust the positive findings for the respiratory tract. It becomes difficult to understand why a vigorous, previously healthy young adult, should succumb to an infection which in many cases appears to be almost wholly confined to the tracheobronchial mucosae. It might even be questioned whether such an individual should die from a *primary* bronchitis and bronchopneumonia. In the opinion of many clinicians and pathologists, such a combination is rather rare.

Because of these considerations and because of a series of observations made on soldiers of the American Expeditionary Forces, it has seemed desirable to suggest that in addition to the infection of the respiratory tract per se, other lesions, almost uniformly neglected by writers on the subject and rarely noted by clinicians or pathologists, may prove to be very seriously important factors both in the clinical picture and in the often fatal outcome of the disease.

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My attention was first directed to the serious nature of so-called "influenzal purulent bronchitis," by a visit in October, 1917, to the base hospital zone of the British Expeditionary Forces in France, at which time Captain Rolland of the Royal Army Medical Corps mentioned his observations in a series of cases of this disease, which had occurred in epidemic proportions and which was highly fatal. The influenza bacillus seemed without doubt to be the causal agent, though pneumococci and streptococci were often also isolated.

On the advent of cold, wet weather in the American Army zone, severe attacks of bronchitis assumed almost the nature of an epidemic and showed a rather high percentage of fatal cases. On examination of these cases postmortem, one was at once struck by the pathologic syndrome presented, which strongly suggested infection by *B. influenzae*. This suspicion was subsequently confirmed by bacteriologic examination.[1]

It is not my purpose at this time to discuss either the general pathologic or bacteriologic characters of this series of cases. It is the involvement of the sinuses at the base of the skull that deserves especial consideration, and because of the importance of this complication, as well as its apparent frequency and the relatively small amount of attention devoted to the subject in medical literature, this separate communication is considered justifiable.

Infection of the accessory sinuses of the nose and skull have often been noted. As early as 1837, Petrequin[2] stated that frontal sinus involvement produced the headache so often present in the disease. Brochin,[3] in 1884, distinguished the "cephalic" form of influenza by localizations in the nasal fossae, and the frontal and maxillary sinuses. Pfeiffer,[4] however, in his classic descriptions of the etiology and pathology of influenza, does not mention the sinuses. In the same year (1892),

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[1] For the larger part of the bacteriologic results in these cases, I am indebted to the careful, thorough work of Lieut. J. G. Hopkins, M. R. C. His painstaking labors, carried out under the most trying conditions, deserve more than passing mention.

[2] Petrequin: Gaz. méd. de Paris, cited by Brochin (Note 2).

[3] Brochin: La grippe, Dictionnaire encyclopédique des sciences médicales, 1884.

[4] Pfeiffer: Vorlaufige Mitteilungen über den Erreger der Influenza, Deutsch. med. Wchnschr., 1892, No. 2, p. 28; Die Aetiologie der Influenza, Ztschr. f. Hyg. u. Infectionskr., 1892, **13.**

Weichselbaum[5] published the necropsy findings of a series of cases, in six of which he found inflammatory lesions in either the ethmoids or the antrum. In two of these cases the influenza bacillus was recovered from the exudate. Bull[6] has also described sinus involvement as a part of the sequelae of influenza. Wassermann,[7] in a fairly complete study of clinical cases and necropsy material, while confirming Pfeiffer's results, evidently did not encounter or missed the significance of any sinus complications. In a review of the entire subject of influenza, Schrötter[8] also fails to note the relation of the sinuses to pulmonary types of the disease.

In the more recent literature there are occasional references to sinus influenza, notably by Kyle[9] and Coates;[10] but Hübschmann,[11] in a study of 110 cases, and Hammond, Rolland and Shore,[12] in an article based on seventy-one necropsies on patients showing purulent bronchitis, do not discuss at all the question of the sinuses. In short, sinus disease in epidemics of influenza is either totally disregarded or described as an accidental complication or sequela of the infection of the pulmonary tract.

When, therefore, our first fatal case of influenzal tracheobronchitis and bronchopneumonia showed, at postmortem examination, an empyema of both sphenoid cavities, and the pus from these revealed by both smears and cultures typical influenza bacilli, the condition was regarded as an interesting but unusual complication. However, as case after case coming to necropsy showed similar or comparable lesions, the circumstances warranted more careful and detailed study.

The conditions found may best be summarized from the abridged notes of the necropsy examinations.

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[5] Weichselbaum: Beitrag zur Aetiologie und pathologische Anatomie der Influenza, Wien. klin. Wchnschr., 1892, p. 459.

[6] Bull: Multiple Sinus Disease Following Influenza, Med. Rec., New York, 1899, **56**, 336.

[7] Wassermann: Einige Beiträge zur Pathologie der Influenza, Deutsch. med. Wchnschr., 1900, No. 28.

[8] Schrötter: In Kolle and Wassermann's Hand Book.

[9] Kyle: Epidemic Nasal Sinus Disease, Jour. Laryngol., Rhinol. and Otol., 1917, **30**, 366.

[10] Coates: Etiology and Diagnosis of Diseases of the Accessory Sinuses Following Influenza.

[11] Hübschmann: Ueber Influenza, München. med. Wchnschr., 1915, **62**, 1073.

[12] Hammond, Rolland and Shore: Purulent Bronchitis, Lancet, London, 1917, **2**, 41.

Case 1.—J. D., aged 22. Duration of illness, sixteen days.

Lungs: Typical purulent tracheobronchitis and bronchopneumonia.

Sinuses: Sphenoidal sinus completely filled with a thick mucopurulent exudate under some pressure. Mucosae markedly swollen and deeply congested. Posterior ethmoids contained small amount of thin, yellowish fluid. Anterior ethmoids and frontals free.

Bacteria: Influenza bacilli recovered from sphenoidal sinus, lung and liver. Occasional gram-positive cocci in pairs and short chains also present.

Case 2.—C. M., aged 23. Duration of illness, twenty-three days.

Lungs: Typical purulent tracheobronchitis and bronchopneumonia.

Sinuses: Left sphenoid filled by thick mucopurulent exudate under pressure. Both posterior ethmoids contained moderate amounts of pus. Mucosae swollen and reddened. Other sinuses free.

Bacteria: Cultures revealed *B. influenzae* in ethmoid, lung, bronchi and liver. Streptococcus also found in bronchus but not in ethmoid.

Case 3.—M. C., aged 27. Duration of illness, twenty-six days.

Lungs: Purulent tracheobronchitis and bronchopneumonia. Mucosae swollen.

Sinuses: Both sphenoids and posterior ethmoids full of thick, mucopurulent exudate. Mucosae swollen. Anterior ethmoids and frontals apparently clear.

Bacteria: *B. influenzae* recovered in pure culture from the sphenoidal pus and the lung, and in mixed culture from bronchi, spleen, liver and bronchial lymph nodes.

Case 4.—L. S., aged 23. Duration of illness, thirty-two days.

Lungs: Purulent tracheobronchitis and bronchopneumonia with fibrinopurulent pleuritis.

Sinuses: Sphenoidal, posterior ethmoidal and frontal sinuses filled with thick mucopurulent exudate. Mucosae swollen and reddened. Anterior ethmoids free but mucosae swollen.

Bacteria: Influenza bacilli from frontal, sphenoid, bronchus, lung and liver Pneumococci also in sphenoid and lung. From bronchus, practically pure culture.

Case 5.—M. H., aged 24. Duration of illness, twenty-nine days.

Lungs: Purulent tracheobronchitis, bronchopneumonia and fibrinopurulent pleuritis.

Sinuses: Mucosae of sphenoidal, ethmoidal and frontal sinuses swollen and reddened. In left sphenoid and both frontals were a few drops of mucopurulent exudate. Other surfaces were bathed by a mucoid fluid.

Bacteria: *B. influenzae* recovered from sphenoid, ethmoid, lung, bronchus and liver. Streptococci also found in sinuses.

Case 6.—A. C., aged 22. Duration of illness, twenty-nine days.

Lungs: Purulent tracheobronchitis, bronchopneumonia and fibrinopurulent pleuritis.

Sinuses: Sphenoids contained a few drops of thin mucopurulent exudate. Other sinuses free. Middle ears and mastoids on both sides showed thick pus.

Bacteria: Influenza bacilli obtained from sphenoid, middle ear, lungs and bronchi.

Case 7.—W. H., aged 22. Duration of illness, twenty-three days.

Lungs: Purulent tracheobronchitis with bronchopneumonia and fibrinous pleuritis.

Sinuses: Sphenoids, posterior ethmoids and right frontal contained abundant mucopurulent exudate, and mucosae were markedly swollen and congested. Middle ears on both sides showed similar condition.

Bacteria: *B. influenzae* found in sphenoid (associated with streptococci and pneumococci), bronchus, pleura and middle ears.

These cases have certain features in common which distinguish them on the clinical and the pathologic side. A purulent tracheobronchitis with patches of bronchopneumonia definitely identifies them as belonging to the "pulmonary type" of influenzal lesion. However, in each instance either one or several or all the sinuses were involved in what was usually a relatively severe inflammatory process. It should be further noted that the cases all occurred in young men, the ages ranging from 22 to 27 and averaging 23. As is usual with influenza, the duration was comparatively long, averaging over three weeks (twenty-four days), and ranging from sixteen to thirty-two days.

In view of the foregoing facts, especially the long duration, the question now arises as to whether, after all, these sinus infections are not merely sequelae or unimportant complications of the principal lesion, which, it may be contended with much reason, is located in the pulmonary system. In the course of our necropsy experiences, considerable evidence accumulated having some bearing on this point.

The following cases illustrate the presence of the syndrome described above, but with the added difference that death was in all probability due to other and seemingly independent conditions:

Case 8.—R. B., aged 18. Duration of illness, eighteen days. Death due to acute general miliary tuberculosis particularly involving lungs, spleen, liver and brain, and arising from an older tuberculous lesion in a seminal vesicle.

Lungs: Acute purulent tracheobronchitis.

Sinuses: Sphenoids, especially the left, filled with thick, yellowish exudate, and lined by swollen reddened mucosae. Mucosae of ethmoids and frontals thickened and moist, but free from pus.

Bacteria: Influenza bacilli from sphenoids and bronchi.

Case 9.—J. M., aged 24. Duration of illness, three days. Death due to epidemic cerebrospinal meningitis.

Lungs: Purulent tracheobronchitis, with bronchopneumonia and seropurulent pleuritis.

Sinuses: Mucosa of sphenoid swollen, but all sinuses free from demonstrable pus.

Bacteria: *B. influenzae* obtained in culture from sphenoid, lung and bronchus.

Case 10.—G. T., aged 27. Duration of illness, thirty-nine days. Death due to scarlet fever. Complicating measles with terminal seropurulent peritonitis and pericarditis.

Lungs: Acute tracheobronchitis (not purulent), with acute serofibrinopurulent pleuritis.

Sinuses: Sphenoids, ethmoids and frontals contained considerable mucopurulent exudate, and mucosae were swollen. Middle ears both showed thick pus.

Bacteria: From the sinuses and middle ears, *B. influenzae* was obtained in cultures and smears. All the other lesions yielded hemolytic streptococci.

Case 11.—F. P., aged 19. Duration of illness, fifty-three days. Death due to double empyema (pneumococcic).

Lungs: Mucopurulent tracheobronchitis.

Sinuses: Sphenoids filled with a mucopurulent exudate. Mucosae swollen but pale. Ethmoids and frontals contained a small amount of mucoid fluid.

Bacteria: Influenza bacilli obtained in mixed culture from sphenoid. No culture taken of the bronchus.

Case 12.—F. B., aged 22. Duration of illness, eleven days. Death due to empyema and pericarditis complicated by pulmonary abscesses (all streptococcic).

Lungs: Purulent tracheobronchitis (pneumococcus, Type III).

Sinuses: Sphenoids showed swollen gelatinous mucosae, and contained a thin mucopurulent fluid. Remaining sinuses appeared normal.

Bacteria: Influenza bacilli obtained by culture from sphenoid.

Case 13.—H. P., aged 18. Duration of illness, thirty-five days. Death due to double empyema (pneumococcic).

Lungs: Acute purulent bronchitis (no culture made).

Sinuses: Sphenoid sinus filled (under pressure) with thick mucopurulent exudate. Mucosae swollen but pale. Remaining sinuses free.

Bacteria: From sphenoid, *B. influenzae* obtained by culture and smears.

As has been indicated, in these six cases, death was in all probability due to some other than an influenzal infection. What the interrelation was between the various infecting organisms furnishes an interesting speculation. That which concerns us at this time is the presence in all these cases of pathologic conditions in the sinuses in which the exudate harbored influenza bacilli that it is fair to presume were pathogenic. It must also be admitted that in some of the cases influenza organisms were possibly responsible for the conditions found in the bronchi and trachea; but certainly in these regions the pathologic processes were no further advanced than in the sinuses. It would not appear, at least, that the sinuses were necessarily secondarily or terminally invaded as compared with infection of the bronchial tree.

Further evidence is produced by two other cases in which sudden death was due to accidental causes:

Case 14.—A. S., aged 22. Duration of life after accident, 40 minutes. Death due to bullet wound through chest, involving heart and lungs.

Lungs: Bronchi apparently normal.

Sinuses: Mucosae of sphenoids swollen; cavities contained mucoserous exudate.

Bacteria: *B. influenzae* isolated from sphenoid in pure culture.

Case 15.—J. H., aged 30. Duration of life after accident, sixteen hours. Death due to fracture of skull and hemorrhage of brain.

Lungs: Bronchi apparently normal.

Sinuses: Left posterior ethmoid showed a few drops of thick mucopurulent exudate. Mucosae swollen and reddened. Other sinuses apparently free.

Bacteria: *B. influenzae* isolated in pure culture from this pus.

In both of these cases, with no macroscopic evidence of bronchial or tracheal involvement, definite inflammatory conditions associated with pure cultures of the influenza bacillus were found in the sinuses. Here, at least, the infection would appear to have been present in the sinuses before it became of any importance in the pulmonary tract. It might then have been a potential nidus for the future development of lung complications. Even granting that the sinus conditions were sequelae of a previous bronchitis from which the patients had recovered, the menace for recurrences and extensions would probably remain the same. After all, the question of whether the lungs or the sinuses were first affected is relatively immaterial compared to the importance of the facts themselves, namely, that the sinuses may be seriously involved in cases of influenzal bronchitis, and also that this infection of the sinuses may exist independently of any lung disease either as a consequence or a cause.

### COMMENT

It would, of course, be unreasonable to assert that all cases of influenza are accompanied by sinus complications. While it is true that in only one of our cases of influenzal purulent tracheobronchitis coming to postmortem did we fail to find sinus disease also present, it is unfortunate that both in this case as well as in the others of the series the antrum of Highmore was not also investigated. However, the number of cases is altogether too small to justify any sweeping conclusions. They do serve most emphatically to emphasize the importance of the sinuses in the respiratory type of influenza.

This importance is interesting from two standpoints. First, from the standpoint of a local focus of infection, which not only constantly menaces the pulmonary system, only awaiting suitable conditions of exposure and lowered resistance for hostile invasion, but also furnishes continued sources of toxic absorption, not to mention the direct effect on the well being of the patient from the presence of these local conditions. Second, and more important, is the bearing that these local infections have on prophylaxis and treatment. When their attention had been called to the possible constant presence of sinus disease in patients suffering from influenzal bronchitis, the attending physicians adopted local measures of treatment for these conditions, even when their presence could not be diagnosed with any degree of certainty. Local applications to the nasal passages of cocain and epinephrin solutions often resulted in copious discharges of thick, mucopurulent exudate from the sinuses, with marked relief to the patient, such as amelioration of headache and pain in the eyes, as well as definite betterment of the conditions in the bronchi and trachea.

While the general proposition that young, otherwise healthy adults do not die from uncomplicated primary bronchitis or bronchopneumonia is by no means without exceptions, nevertheless, it is always extremely desirable in such cases to search most carefully for foci of infection other than the respiratory tract per se. The middle ears, serous membranes and even the blood stream may harbor the offending organisms, but in cases of influenza and possibly other respiratory diseases,[13] the sinuses of the skull should always be investigated and their freedom from involvement assured before other than general therapeutic measures of relief are abandoned.

Inevitably in these days, the question of carriers is linked up with that of actual cases, and perhaps too little consideration has been given to sinus disease as furnishing chronic foci for the spread of epidemics.

The entire subject deserves further investigation, and whether or not my conclusions are found to be justified, in the study of larger groups of cases and by other workers, is a matter of minor importance. If more careful attention is directed toward what at times is an important feature of epidemic influenza, namely, sinus involvement, this communication will have fully accomplished its purpose.

## SUMMARY

1. Epidemics of respiratory influenza (purulent tracheobronchitis) have been fairly severe in both the American and the British Expeditionary Forces.

2. In the investigation of cases, both clinically and at postmortem, little attention in the past has been given to the question of accompanying sinus disease.

3. Of eight fatal cases of purulent tracheobronchitis due to the influenza bacillus, all but one showed involvement of one or more of the sinuses at the base

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[13] As, for example, whooping cough, which is very closely related to influenza both in the characters of the causative organisms, as well as many of the clinical features of the disease. The spasmodic cough of some influenza patients is often difficult to distinguish from the "whoop" caused by the Bordet-Gengou bacillus.

of the skull by inflammatory processes, probably, in all cases, directly due to the invasion of these sinuses by the influenza bacillus.

4. In six patients that died from some other apparently independent infection, the sinuses showed influenzal inflammations.

5. Of two patients dying from accidentally received injuries, both harbored in their sinuses lesions giving pure cultures of *B. influenzae*.

6. Appropriate treatment of the sinuses in patients suffering from influenza often served to relieve the symptoms and apparently to hasten convalescence.

7. Investigation of the sinuses during epidemics of influenza is strongly recommended and urged not only on therapeutic but also on prophylactic grounds.

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# CEREBRAL HEMORRHAGE OF THE NEWBORN.

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SINCE earliest times the traumatism undergone by an infant during the process of birth has been recognized and deplored. This is, of course, especially marked in long or difficult labors, and we have become only too familiar with the severe injuries which may result from this cause. The most common of these has been naturally head injury, associated with hemorrhages which often have been considered as a cause of stillbirth or death during the first few days of life. But, although recognized as a theory, on reviewing the literature one finds very few statistics founded upon detailed autopsy reports in cases of cerebral hemorrhage of the newborn. Although a subject of vital interest and fundamental importance to the obstetrician, pediatrician and pathologist it seems to belong to none and to be neglected by all.

Little, in 1861, was the first to emphasize birth injuries, and their results in later life, as shown by the development of impaired mentality or the palsies or contractures in older childhood. But as at that time the effects of pressure on the cortex was but imperfectly understood, it was left to Sarah McNutt, in 1885, to establish the relationship between cerebral hemorrhage at birth and Little's disease of later life. This was done by autopsy reports on 10 carefully studied cases. Of these 10, 7 were vertex and 3 breech presentations, showing that prolonged pressure on the head is not always responsible for the condition. Of the 7 vertex presentations 5 showed hemorrhage over the cerebrum and 2 below the tentorium. The hemorrhage in the breech presentations was over the cerebrum in all 3.

Of the occurrence of cerebral hemorrhage as compared with other causes of death in infants only meager information is found Holt says that one-third of all deaths during or as the result of parturition are due to hemorrhage, but gives no exact numbers based on autopsy findings. Cushing found that in studying stillborn infants for another purpose, many showed cerebral hemorrhage. From the Pathological Institute of Kiel, Weyhe reported that in 959 autopsies on infants 122, or 12 per cent., showed intracranial hemorrhage. In 80 of these cases the hemorrhage was subdural, in 56 subarachnoidal, in 35 intracerebral and in 21 intraventricular. Of these 122 cases 23 showed evidence of congenital syphilis. Döhle found hemorrhages occurring in 13.7 per cent. of his series. Beneke observed in 100 autopsies on the newborn laceration of the tentorium resulting in hemorrhage in 14 cases. Osler quotes Litzmann as finding 35 cases of hemorrhage in 161 autopsies, Parrot as noticing bleeding in the subdural space 26 times in 34 autopsies of the newborn and Spencer finding in 130 postmortem examinations on stillborn infants 53 cases with hemorrhage under the pia-arachnoid. Sachs in autopsies on 78 cases of infantile hemiplegias noticed hemorrhage in 23. Couvelaire saw intracranial bleeding in 18 per cent. of autopsies on prematures born with easy and spontaneous labors. Archibald reports in 74 postmortem examinations that intermeningeal hemorrhage occurred in 32, and this hemorrhage was extensive in 19 and extradural in 5.

Isolated cases confirmed at the autopsy table are quite numerous in the recent literature, especially since Cushing's article describing operative procedure for the relief of the condition. Green describes 1 with blood over both hemispheres and at the base along the crura and pons, 1 other with clots over the right hemispheres and 2 more with bleeding over the cerebrum accompanied by hemorrhages in other organs. Eastman reports 1 case with bleeding in both ventricles. Torbert had 2 cases, both with hemorrhage over the cerebrum. Simmons saw 2 cases with the same distribution. Others are given by Cushing and Meara and Taylor.

Every pathologist familiar with autopsies on the newborn has noticed, even without massive bleeding in any organ, numerous small punctate hemorrhages occurring especially over the epicardium and the parietal pleura. Paul finds these same hemorrhages in the retinæ of the newborn during life. In 200 examinations he noted hemorrhage in the retina in 20 per cent. of children born with a normal labor, 50 per cent. of prematures and 40 per cent. of those from complicated and prolonged labors. In view of these findings the assumption is justifiable that young infants' bloodvessels are particularly delicate and susceptible to rupture from injury.

As to the etiology of these hemorrhages one finds as many theories suggested and advanced as there are authors. All agree, however, that the condition is as frequent in cases of normal or precipitate delivery as in difficult labors. It has also been shown that hemorrhage may occur even before birth. Gibb reports a child born with contractures of one arm and leg and an old blood clot above the lateral ventricle, while the mother gave a history of trauma. Osler found in an autopsy on a woman dying of typhoid in the seventh month of pregnancy a fetus containing a large hemorrhage in the brain. Seitz quotes another case in which a macerated fetus delivered spontaneously had a hematoma the size of a walnut in the brain hemisphere.

Kreyberg thinks that a long time elapsing between the birth of the head and shoulders is an etiological factor, while La Letra states that hemorrhage frequently follows asphyxia caused by the cord being twined about the neck. Benthin suggests that it may be caused by too great pressure on the perineum. Beneke points out that during severe labor pains the pressure is often on the sides of the head, increasing its long axis, pressing the brain forcibly against the tentorium and causing tearing of its radiating fibers. He further explains that this tear may be of the anterior, posterior or middle layer, and therefore may cause bleeding over either the cerebrum or cerebellum. But Seitz, after a careful study in which he cut serial sections of the head and its contained brain, concluded that although one-half of his cases confirmed Beneke's theory the other one-half excluded tentorium injuries as a cause and pointed instead to injuries of the vessels over the brain. He therefore groups all of these cerebral hemorrhages into three divisions: (1) hemorrhage over the cerebrum (one or both hemispheres) from injury to the longitudinal sinus or the vessels emptying into it; (2) hemorrhage under the tentorium around the cerebellum, due to injuries to the transverse sinus or tributary vessels; (3) hemorrhage into the lateral or fourth ventricles, due to the tearing of the veins of the choroid plexus. The last group is evidence against the theory of tentorium injury being the only cause of cerebral bleeding.

Abels places the causes under two groups: (1) Tearing of the vessels due to molding of the head in such cases, as contracted pelvis, forceps deliveries, etc.; (2) backing up of the blood in the sinuses and veins in asphyxia. He points out that during labor the pressure on the fetus is raised in the uterus, and especially before the membranes are ruptured, is evenly distributed, thus causing no congestion or stasis of the blood in the child's head. But when the head is born it is released from the uterine pressure still acting on the body, and the result is a marked congestion or stasis of the bloodvessels of the head. Since the skull cannot expand to any marked degree the delicate-walled veins on the surface of the brain may become extremely engorged and often rupture. The sutures permit of some expansion, and Abels thinks that this is the reason hemorrhage is rarely found in the neighborhood of the sutures.

Cushing adopts the same classification as Abels and says that rupture may be possible in marked asphyxia, just as it may be possible in later life in a paroxysm of whooping-cough or a convulsion. He also notes the frequency of rupture of the veins at the point of entrance into the longitudinal sinus and points out that in infants there are no adhesions between the dura and cerebral hemispheres, thus leaving these veins unprotected and in a position to be easily displaced or torn by the overriding of the parietal bones during the molding of the head.

Seitz made an effort to find the etiology of cerebral hemorrhage in the newborn by making a careful analysis of the 13 cases which came under his observation. Of these infants he found 5 to be below and 8 above average weight, suggesting oversize as a possible etiological factor. Of the 13 mothers 7 were primipara, and of these 4 were over thirty years of age; 6 were multipara, and of these 4 had borne many children. One-fourth of the mothers had a contracted pelvis, but in none was it marked. Of the 13 deliveries forceps were used 10 times, leaving only 3 spontaneous births; 11 of the 13 babies were born asphyxiated, 5 to a mild degree and 6 to a marked degree. However, of the 3 unaided deliveries 2 breathed spontaneously, all 3 mothers were multipara, none had narrow pelves, none had lues, the births were all rapid and the cervices all quickly and completely dilated. In view of these facts the explanation of the occurrence of the hemorrhages becomes exceedingly difficult or obscure. But all three babies were large, so Seitz reaches the conclusion that although dilatation appeared complete it was not sufficient for children so large, and in the rapid delivery the head was too quickly molded and injury to the veins was the result.

All authors agree on the predominance of bleeding over the cerebrum, usually limited to one side. It is also frequently pointed out that the child may appear normal for the first few days and then develop cerebral symptoms. Kundrat thinks that many of these infants recover from hemorrhage resulting from birth trauma without developing any symptoms, for in autopsies on children several weeks old he has found old pigment in the meninges and other signs of old extravasated blood. Meara and Taylor warn against "jackknifing" as a means of artificial respiration, on the ground that it increases the distention of the cerebral vessels already engorged by the asphyxia. This assumption is given greater weight by the occasional discovery of subdural and extradural hemorrhage in the spinal column following such manipulations. But to Green belongs the credit of first suggesting hemorrhagic disease of the newborn as a cause of cerebral hemorrhage.

In the pediatrics service at the University Hospital during the last three years 36 routine autopsies have been performed on stillborn babies or those dying early in infancy. In this series 18, or 50 per cent., showed definite hemorrhages in the dura, over the brain or in the ventricles. Of the 18, 10 occurred in females and 8 in males. If one takes Seitz's figure of 3200 grams as the average weight of a full-term child, of our 18 cases 11 were of average weight or below and only 7 above. Of the 18 mothers 11 were primiparæ of these 11 only 1 was over thirty years, 1 was twenty-nine and the remaining 9 were twenty-four or under. Of the 7 multiparæ 2 were second labors, 1 was third, 1 was fourth, 1 was fifth, 1 was eighth and 1 was ninth. Forceps were used but once, and then in the case of delivering a mother, critically ill with pneumonia, of a six months' fetus. In only 2 cases was the labor very long, and in both of these cases twins were born, in each instance the first one remaining normal while the second showed cerebral hemorrhage. Of the 18 babies only 2 were born dead and 4 exhibited signs of asphyxiation, while the remaining 12 breathed spontaneously, thus excluding mechanical asphyxia as a major factor in this condition. Only 2 infants appeared normal at and following birth, the others showing respiratory symptoms from the very first.

One very interesting point, however, is that in 8 of these infants gross hemorrhages were found in other organs besides the brain. It thus appears quite clear that in a fairly large percentage of cases showing intracranial hemorrhage such lesions are merely incidental to a general hemorrhagic diathesis, the syndrome being usually grouped under the heading, "Hemorrhagic Disease of the Newborn." Five of the 8 vomited blood before death and showed an increased coagulation-time of the blood. Of the 3 remaining cases without clinical symptoms 1 revealed besides the hemorrhage over the brain an extravasation of blood into the right adrenal, another, massive hemorrhages in the lungs and the third hemorrhages in the pericardium and skin. These facts emphasize the value of routine estimations of the coagulation-time on the blood of newborn infants. Early recognition of decreased coagulability of the blood and prompt treatment of such cases by the injection of whole blood or serum might obviate otherwise fatal hemorrhages in the brain as well as other organs. Of the two small twins each born alive after its fellow the condition cannot be explained on the ground of overweight or of only partially dilated cervix, but one showed massive hemorrhage in the lungs as well as over the brain and the other was definitely, clinically and anatomically hemorrhagic disease.

Kundrat points out that partial atelectasis of the lungs is always associated with cerebral hemorrhage and is almost pathognomonic of it. While our necropsy findings show that the two conditions are associated, atelectasis of varying degrees was also found in infants not showing cerebral hemorrhage. Hence, as partial atelectasis may be explained by the short period of life, asphyxiation or prematurity, it may be considered as, at the most, merely a contributory and not as a primary condition.

The location of the hemorrhages of the series of cases from the University Hospital conforms well with other observations given above. In this series hemorrhage over the cerebrum and limited to one hemisphere occurred six times; over the cerebrum, cerebellum and in the dura three times; over the cerebrum, cerebellum and in the ventricle once; over the cerebrum and cerebellum three times;

over the cerebellum once; in the dura alone only twice; and in the ventricles alone twice. Hemorrhage over the cerebrum either alone or with other parts of the brain is found in a total of 13 cases, or 72 per cent. In only 2 cases was softening of the brain under the hemorrhage noted, and this occurred in the oldest in the series, an infant of five days, and in a premature one month of age. In the other cases doubtless death occurred before degenerative changes could take place in the brain substance. In no case was gross hemorrhage into the brain substance found. None of these infants showed any signs of congenital syphilis, and only one mother gave a positive Wassermann reaction. Only four were born prematurely. The high percentage of hemorrhages in these cases may be partially due to the fact that very few emergency or acutely ill patients are admitted to the University Hospital, so that the list therefore includes few acute diseases of either mothers or infants and is limited to births occurring in the hospital.

When confronted by the resumé of the literature and the analysis of the cases give above, one is impressed by the comparative frequency of occurrence of cerebral hemorrhage in newborn infants and also by the number and confusion of the etiological factors claiming attention. A classification of the various causes which have been proposed will emphasize this latter fact and also form a basis for a discussion of their relative merits. They may readily be divided into three main groups:

I. Traumatic: from molding the head, either in normal or precipitate deliveries.

1. Over the cerebrum:
    - (*a*) Due to injury of the longitudinal sinus or its tributary vessels.
2. Below the tentorium:
    - (*a*) Due to injury of the transverse sinus or its tributary vessels.
3. In the ventricles:
    - (*a*) Due to injury of the choroid plexus.
4. In the dura.
    - (*a*) Due to elongation of the head with pressure of brain against dura and tentorium.

II. Congestion or stasis with rupture of veins in protracted or complicated labor due to:

1. Malpresentations.
2. Overgrowth of child.
3. Twins.
4. Umbilical cord around neck.
5. Rigid cervices in primipara.
6. Prolongation of the interval between the birth of the head and that of the shoulders.

III. A diseased condition of the child in intra-uterine life and having no relationship to labor, such as:

1. Hemorrhagic disease of the newborn.
2. Prematurity.
3. Syphilis.
4. Congenital heart disease.
5. Other toxemias.

Considering the subject as a whole it becomes at once clear that the most frequent source of this bleeding, regardless of cause, is rupture of the superficial veins which pass over the surface of the brain into the longitudinal sinus. This may be explained by the fact that these veins are in a comparatively unprotected position, and hence more susceptible to injury from either displacement or abnormal distention. Of all vessels in the body these are the ones most exposed to the injuries and accidents of labor and birth.

The first division of the above classification represents, theoretically at least, one of the most common causes of injury, *i. e.*, overriding of the parietal bones and resulting displacement and laceration of the veins. For this condition even an abnormal labor is not necessary, and it frequently is most marked in normal deliveries, especially those which are rapid or precipitate. In fact, the more rapid the delivery the less chance do the brain and vessels have to adjust themselves to the changed relationships and the greater tendency there is to injury. This point is emphasized in the 18 cases cited above in which only 2 showed a prolonged labor. In the overriding of the parietal bones the condition is naturally the most marked on one or the other side, and as a result the hemorrhage is frequently limited to one cerebral hemisphere, as was noted in 6 cases of our series. Of less moment, perhaps, but following the same principles, the changes in the relation of the occipital bone to the parietals may account for hemorrhages over the cerebellum, and perhaps in certain cases even those originating from rupture of the choroid plexuses. The hemorrhages in the dura only may be accounted for by injury to the tentorium or dura, either by laceration of their fibers or of their capillaries in the twisting or elongation to which the head is subjected in its passage through the birth canal. The use of forceps is often advanced as an explanation of these hemorrhages, but one finds in the 18 cases analyzed above that forceps were employed but once, and then in a very easy delivery. There is little doubt that this factor has been greatly overemphasized. Their usual position in this category may be due to a confusion of the ideas of cause and effect. The harm is doubtless done in many instances not by the mechanical injury of the forceps themselves, but by congestion or asphyxiation due to the protracted or complicated labor which caused the attempt at artificial aid to delivery.

The second group includes those cases in which labor is prolonged and the resulting congestion and overdistention of the veins lead directly to hemorrhage or indirectly to rapid degeneration and subsequent rupture, just as in later life undue dilatation of a cerebral vessel may result from a paroxysm of whooping-cough or a convulsion. Narrow pelves, malpresentations or an abnormally large child are self-explanatory factors in producing mechancial obstruction to normal birth of the infant. The constriction of the neck by encircling with the umbilical cord is also a fairly common cause of asphyxiation. A long time elapsing between the birth of the head and that of the shoulders may result in a marked congestion of the cerebral veins, due to the fact that while the head is free the body is still subjected to uterine pressure and the blood is forced out of the body into the head. In the case of twins the first child may be normal while the second, even though delivered quickly, will show marked congestion because of too long delay in birth. In respect to primipara it is interesting to note that in the cases above cerebral hemorrhage occurred more frequently in young primipara mothers than in older ones. The difficulty here is doubtless due to rigidity of the cervix, with resulting slow and imperfect dilatation in the first labor, and this occurs in young as well as older women.

In the third group of cases the cause of the hemorrhage undoubtedly is in the fetus and has little or no relationship to the process of labor itself or the conditions accompanying birth. In this division hemorrhagic disease of the newborn deserves by far the most important place because of the comparatively large number of deaths with cerebral hemorrhage showing this condition. It accounts for the death of 8 of the 36 cases in our series. One is justified in emphasizing the importance of this disease because of the fact that it has been largely disregarded in the past. Prematurity, while not an actual disease, is placed here because it represents a pathological condition of the child rather than of the mode of birth. Underdevelopment of the body as a whole naturally includes abnormally delicate bloodvessel walls and hence greater liability to rupture from any cause. Moreover, the conditions which have produced a premature birth are often those which have also brought about pathological changes in the tissues, including the bloodvessels, and this has increased the possibility of accident. It is important to note in this connection that nearly one-fourth of the autopsies in our series of cerebral hemorrhages were on premature infants.

Syphilis is perhaps given undue importance as an etiological factor. In none of the 18 cases of our series was there any evidence of this disease. Weyhe claims a place for it, and the importance of syphilitic degeneration of the vessel walls cannot be denied as a possible cause of early rupture. As a rule, however, the disease itself usually accounts for the death of the child and cerebral hemorrhages do not appear to be a common complication. The influence of congenital heart disease, particularly those forms which interfere with the development of a normal extra-uterine circulation and the resulting abnormal congestion thus produced, is a self-evident cause of the occasional appearance of complicating and intracranial hemorrhage. This point is well illustrated in one of our series in which the heart presented an absence of the interventricular septum, and from this common ventricle only one arterial trunk carried away the blood destined for both pulmonary and systemic circulation, the mixture of which must unavoidably lead to profound asphyxiation.

Lastly under this heading must be placed certain cases in which the exact cause of the hemorrhage is by no means clear but in which a diverse group of intoxications occurring in the mother may reasonably be assumed as having an important relation to antenatal degeneration and weakening of the vessel walls. An example of this was, in our series, the small six months' fetus, quickly delivered from a mother dying of lobar pneumonia, which showed marked cerebral hemorrhage.

In conclusion, a general consideration of cerebral hemorrhage in newborn infants emphasizes two very important facts: (1) that the condition is often a complex disease syndrome giving rise to diverse clinical symptoms, modes of death and pathological findings, and (2) that it is not brought about by any single cause but by an interrelation and interaction of a varying number of causes which may be found in the circumstances governing labor in the condition of the mother or of the child. Several causes may often be present, any one of which in itself might have been the responsible factor.

SUMMARY. 1. Cerebral hemorrhage of the newborn is frequently found, occurring in 50 per cent. of 36 deaths of young infants at the University Hospital.

2. The condition is brought about by trauma in normal or rapid deliveries, by congestion or asphyxiation in slow deliveries or by disease of the child itself.

3. The so-called "hemorrhagic disease of the newborn" is a much neglected but very important cause of cerebral hemorrhage in infants, occurring in 44 per cent. of the deaths of our series.

4. Forceps deliveries, advanced age of the primipara mother and syphilis probably do not play as important a role in the etiology of this condition as was formerly supposed.

5. More careful and complete routine autopsies on newborn infants as well as more accurate observations on the conditions of the mothers and circumstances of the birth are needed as a foundation for further studies.

I am indebted to the department of pediatrics for obtaining permission for these autopsies and to the department of obstetrics for their careful records on their parturition charts.

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